ARCHIVES
OF
THE MIDDLESEX HOSPITAL
VOLUME XXIII

# Tenth Report
FROM THE
Cancer Research Laboratories

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ARCHIVES

OF

THE MIDDLESEX HOSPITAL

Tenth Report

FROM THE

Cancer Research Laboratories

(BEING THE TWENTY-THIRD VOLUME OF THE ARCHIVES).

EDITED FOR THE CANCER INVESTIGATION COMMITTEE

BY

W. S. LAZARUS-BARLOW, M.D., F.R.C.P.,

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NOTICE.

In the following Pages, excepting where an asterisk (*) *is placed, or where the context makes it clear that such is not the case,* EVERY DIAGNOSIS OF MALIGNANT DISEASE HAS BEEN MADE AS THE RESULT OF MICROSCOPICAL EXAMINATION.

W. S. L.-B.

REPORTS

FROM THE

CANCER RESEARCH LABORATORIES

TABULATED SYNOPSES OF THE POST-MORTEM EXAMINATIONS AND OPERATIONS IN CASES OF MALIGNANT DISEASE DURING THE YEAR 1910.

By THE DIRECTOR and his ASSISTANTS.

In the following tables are given the results of all cases of malignant disease, as determined by microscopical examination, which were investigated in the Cancer Research Laboratories during the year. The material was derived partly from the post-mortem room and partly from the operating theatre. In all 236 cases of malignant disease (males 56, females 180) have been examined microscopically. All of the above were in-patients. The total number of admissions to the hospital as in-patients during 1910 was 4,357; viz., 2,160 males, and 2,197 females. In addition 12 males and 40 females with malignant disease were admitted to the electrical (out-patient) department for X-ray treatment. Histological examination was not made in these cases.

Besides the cases that have been mentioned, a certain number of patients were admitted (either to the general wards or to the special wards) in which the diagnosis was not made certain by histological examination. These are grouped in two classes according to the relative probability of accuracy in the diagnosis.

In the first group, the diagnosis was founded on naked-eye appearances or on touch, but the patients were either discharged unrelieved from the Hospital at their own request, or else left after palliative or exploratory operation (*e.g.*, cases of gastrostomy, colotomy, &c.).

In the second group the diagnosis was made upon clinical grounds alone.

GROUP I.

Cases diagnosed as Malignant Disease on Evidence derived from the Naked-Eye Appearance, or Touch.

Site.	1910.		
	Males.	Females.	Total.
Breast	1	45	46
Uterus	—	34	34
Ovary	—	1	1
Vulva	—	2	2
Bladder	3	1	4
Pancreas	1	3	4
Mouth	4	—	4
Lip	3	—	3
Tongue	10	1	11
Sup. Maxilla	—	1	1
Palate	2	—	2
Tonsil	2	1	3
Stomach	6	2	8
Rectum	17	11	28
Prostate	4	—	4
Penis	2	—	2
Colon	—	2	2
Gall-bladder	1	1	2
Abdomen (sarcoma)	1	—	1
Pylorus	—	1	1
Neck	—	1	1
Face and Neck (sarcoma)	1	—	1
Humerus (sarcoma)	1	—	1
Orbit (sarcoma)	—	1	1
Pharynx	1	—	1
Thyroid	1	—	1
Cheek	1	—	1
Left scapula and shoulder	—	1	1
Totals	62	109	171

GROUP II.

Cases diagnosed on Clinical Evidence only.

Site.	1910.		
	Males.	Females.	Total.
Œsophagus	15	3	18
Stomach	11	10	21
Peritoneum	—	3	3
Liver	5	1	6
Sigmoid	3	2	5
Pylorus	2	—	2
Intestine	—	2	2
Abdomen	1	—	1
Gall-bladder	1	—	1
Pelvis	—	1	1
Totals	38	22	60

TABLE I.—

—		Total.		0—5.		6—10.		11—15.		16—20.		21—25.		26—30.		31—35.	
		M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
CARCINOMA.																	
Face	Squamous	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Tongue	Squamous	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mouth	Squamous	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pharynx	Squamous	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Larynx	Squamous	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Œsophagus	Squamous	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Stomach, Body ...	Spheroidal	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Columnar	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
„ Pylorus ...	Spheroidal	1	1	—	—	—	—	—	—	—	—	—	—	1	—	—	—
Cæcum	Spheroidal	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Colon	Columnar	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sigmoid	Columnar	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rectum	Columnar	—	3	—	—	—	—	—	—	—	—	—	—	—	1	—	—
Gall-bladder	Columnar	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pancreas	Spheroidal	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Columnar	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Adrenal	Hypernephroma.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Urinary bladder ...	... ...	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Uterus, Body	Columnar	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
„ Cervix ...	Squamous	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	2
Vulva	Squamous	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Breast	Spheroidal	—	14	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ovary	Spheroidal	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Columnar	—	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CARCINOMA AND SARCOMA ...		—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SARCOMA.																	
Mixed-cell		1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Small round-cell		1	2	—	—	—	—	—	—	—	groin	—	—	lung	—	—	—
Chloroma		—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—
ENDOTHELIOMA		—	7	—	—	—	—	—	—	—	—	—	—	—	—	—	pleura
HISTOLOGY DOUBTFUL		2	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		20	69														

Post-mortem Cases.

36—40.		41—45.		46—50.		51—55.		56—60.		61—65.		66—70		71—75.		76—80.		81—85.		86—90.	
M.	F.	M.	F.	M.	F.	M.	F	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
—	—	—	—	—	—	—	—	—	2	—	—	—	1	—	—	—	—	—	—	—	—
—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
—	—	—	—	1	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	1	—	—	1	1	—	—	—	—	—	—	—	—
—	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	1	—	—	1	1	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—
—	—	—	—	—	1	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—
—	—	—	1	—	—	—	3	—	—	—	3	—	1	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	1	—	2	—	3	—	2	—	4	—	1	—	—	—	—	—	—	—	1
—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	1	—	1	—	3	—	—	—	1	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	cervix uteri	—	—	—	—	—	—	—	—	—	—
—	—	neck	—	—	—	—	—	—	uterus	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	bladder	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	cervix uteri	—	—	—	cervix uteri	—	—	—	cervix uteri, jaw	—	breast	—	—	—	—	—	breast	—	—	—	—
—	—	—	—	—	—	bladder	vulva, cervix uteri	—	uterus (2)	—	—	—	—	bladder	—	—	—	—	—	—	—

TABLE II.—

		Total.		0—5.		6—10.		11—15.		16—20.		21—25.		26—30.		31—35.	
		M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
CARCINOMA.																	
Upper Jaw ...	Squamous	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lip	Squamous	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Tongue	Squamous	5	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Palate	Squamous	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pylorus	Spheroidal	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Intestine ...	Spheroidal	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Columnar	3	7	—	—	—	—	—	—	—	—	—	—	—	sigmoid	—	—
Uro-genital System ...	Squamous	5	15	—	—	—	—	—	—	—	—	—	—	penis	cervix uteri	—	cervix uteri
	Columnar	—	10	—	—	—	—	—	—	—	—	—	—	—	ovary	—	—
	Hypernephroma	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Breast	Spheroidal	—	42	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Columnar	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Other Sites ...	Squamous	6	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—
RODENT CANCER		1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SARCOMA.																	
Mixed-cell		1	3	—	—	—	—	—	—	—	—	—	—	—	—	groin	—
Small round-cell		2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Spindle-cell		—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	spinal meninges
Chondrosarcoma		1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Melanotic		—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
ENDOTHELIOMA		—	6	—	—	—	—	—	—	—	—	—	—	—	breast	—	—
DOUBTFUL		3	12	—	—	—	—	—	—	cervical glands	—	—	—	-	—	—	—
		37	110	TOTAL NUMBER OF HOSPITAL OPERATION CASES FOR MALIGNANT DISEASE													
NON-MALIGNANT.		13	27	2	—	—	—	—	—	2	1	2	1	—	2	1	1
		50	137	TOTAL NUMBER OF HOSPITAL OPERATION CASES EXAMINED IN THE CANCER RESEARCH LABORATORIES.													

Carcinoma—Males 30, Females 85
Sarcoma „ 4, „ 7
Endothelioma „ 0, „ 6
Doubtful „ 3, „ 12

OPERATION CASES.

36—40.		41—45.		46—50.		51—55.		56—60.		61—65.		66—70.		71—75.		76—80.	
M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
-	—	—	--	—	—	--	—	—	—	—	1	—	—	—	—	—	--
—	—	1	—	1	—	—	-	2	—	3	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	1	—	3	—	—	—	1	1	—	--
—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	--
—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	-	—	—	—	rectum	—	—	—	—	—	—	—	rectum
—	rectum	—	—	—	—	—	colon	—	sigmoid (2)	rectum (2)	rectum	rectum	—	—	rectum	—	—
—	cervix uteri (6)	—	cervix uteri, vulva.	—	cervix uteri	scrotum	cervix uteri	bladder	cervix uteri, vulva.	penis	cervix uteri.	—	—	penis	—	—	—
—	cervix uteri	—	cervix uteri(2)	—	ovary	—	—	—	ovary	—	cervix uteri, fundus uteri (3)	—	—	—	—	—	—
—	—	—	—	—	--	—	—	1	—			—	—	—	—	—	—
—	4	—	8	—	9	—	1	—	9	—	5	—	2	—	1	—	—
—	1	—	—	—	—	—	--	—	—	--	—	—	1	—	--	—	—
face	—	face	face	—	-	face	—	face	face	—	—	hand	scalp	—	—	face	skin of abd. wall
1	—	—	—	—	--	--	—	—	—	—	1	—	—	—	—	—	—
—	ovary	—	—	—	breast	—	—	—	—	—	uterus	—	—	—	--	—	—
—	—	testis	—	rectum	—	—	breast	—	—	—	--	—	breast	—	--	—	--
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
testis	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	-	—	—	—	—	--	—	vulva	—	—	—	—
—	breast	—	breast	—	—	—	omentum, breast	—	—	—	cervix uteri	—	—	—	—	—	—
anal canal, skin	breast (2), skin	—	—	.—	cervix uteri	—	cervix uteri (2), ovary, breast.	—	breast(2) cervix uteri	--	—	—	—	—	—	—	skin
1	6	--	3	—	4	2	2	—	3	1	3	2	1	—	—	—	—

TABLE III.

SYNOPSES OF POST-MORTEM CASES.

CARCINOMATA.

No	Initials and Cancer Register Number.	Sex.	Age at Death.	Nature of new growth and part primarily affected.	Sites of Secondary new growth.	Other morbid changes present.	(i.) Congenital abnormalities. (ii.) General remarks.	(i.) Date of admission. (ii.) Date of death. (iii.) Surgical operation, if any.
1	A.S. 176/10	F	57	Squamous cell carcinoma of face.	Cervical glands.	... ...	(ii) Emaciated.	(i) 8 July '10. (ii) 9 Aug. '10.
2	E.C.D. 136/10	F	59	Squamous cell carcinoma of face.	None.	... ...	(ii) Well nourished.	(i) 6 May '10. (ii) 29 June '10.
3	A.F.A. 284/10	F	70	Squamous cell carcinoma of face.	None.	... ...	(i) Patent foramen ovale. (ii) Some emaciation.	(i) 7 May '10. (ii) 8 Dec. '10.
4	W.M. 292/10	M	47	Squamous cell carcinoma of tongue.	None.	... ...	(ii) Well nourished. Hosp. p.m. 234.	(i) 28 Nov. '10. (ii) 15 Dec. '10.
5	H.S. 301/10	M	64	Squamous cell carcinoma of floor of mouth.	None.	... ...	(ii) Great emaciation.	(i) 17 June '10. (ii) 24 Dec. '10.
6	W.H.R. 81/10	M	46	Squamous cell carcinoma of pharynx.	Thyroid, cervical glands.	... ...	(ii) Well nourished.	(i) 22 Dec. '09. (ii) 4 April '10.
7	J.M. 191/10	M	58	Squamous cell carcinoma of pharynx.	None.	Acute pericarditis. Left pleural effusion.	(ii) Emaciated.	(i) 9 June '10. (ii) 20 Aug. '10.
8	G.P. 266/10	M	40	Squamous cell carcinoma of larynx. Multiple endotheliomata of intestine.	Cervical glands.	Broncho-pneumonia.	(ii) Emaciated.	(i) 10 Dec. '09. (ii) 10 Nov. '10.

9	R.Y. 175/10	M	52	Squamous cell carcinoma of œsophagus, middle third.	None.	... ...	(ii) Emaciated. Hosp. p.m. 149.	(i) 3 Aug. '10. (ii) 9 Aug. '10.
10	J.B. 5/10	M	68	Squamous cell carcinoma of œsophagus, lower third.	Liver, kidney.	Abscess of lung.	(ii) Emaciated. Hosp. p.m. 3.	(i) 10 Dec. '09. (ii) 7 Jan. '10.
11	F.G.L. 30/10	M	28	Spheroidal cell (colloid) carcinoma of body of stomach.	None.	... ...	(i) Meckel's diverticulum. (ii) Great emaciation. Hosp. p.m. 19.	(i) 26 Jan. '10. (ii) 2 Feb. '10.
12	J.B. 198/10	F	51	Spheroidal cell carcinoma of body of stomach.	Liver.	... ...	(ii) Emaciated. Hosp. p.m. 161.	(i) 5 Aug. '10. (ii) 30 Aug. '10.
13	J.E. 171/10	M	52	Columnar (becoming transitional) cell carcinoma of body of stomach.	Liver.*	Ascites.	(ii) Much wasted. Hosp. p.m. 145.	(i) 16 July '10. (ii) 5 Aug. '10.
14	W.M. 122/10	M	53	Columnar cell carcinoma of body of stomach.	Pancreas, portal glands.	... ...	(ii) Some emaciation.	(i) 6 April '10. (ii) 7 June '10. (iii) Exploratory laparotomy. Gastro-enterostomy.
15	M.O'C. 104/10	F	54	Columnar cell carcinoma of body of stomach.	None.	... ...	(ii) Some emaciation.	(i) 19 Feb. '10. (ii) 10 May '10.
16	H.F. 89/10	F	62	Spheroidal cell carcinoma of body of stomach.	None.	... ...	(ii) Fair nutrition. Hosp. p.m. 83.	(i) 9 April '10. (ii) 15 April '10.
17	J.B. 134/10	M	49	Spheroidal cell carcinoma of pylorus.	None.	Gangrene of lung.	(ii) Fair nutrition. Hosp. p.m. 116.	(i) 9 June '10. (ii) 28 June '10.
18	E.G. 164/10	F	60	Spheroidal cell carcinoma of pylorus.	None.	... ...	(ii) Fair nutrition. Hosp. p.m. 143.	(i) 27 July '10. (ii) 1 Aug. '10.

TABLE III.—SYNOPSES OF POST-MORTEM CASES—*cont.*

No.	Initials and Cancer Register Number.	Sex.	Age at Death.	Nature of new growth and part primarily affected.	Sites of secondary new growth.	Other morbid changes present.	(i.) Congenital abnormalities. (ii.) General remarks.	(i.) Date of admission. (ii.) Date of death. (iii.) Surgical operation, if any.
19	V.D. 22/10	M	45	Spheroidal cell (colloid)carcinoma of cæcum.	None.	... ...	(ii) Body stout. Hosp. p.m. 13.	(i) 20 Jan. '10. (ii) 22 Jan. '10.
20	S.T. 54/10	F	56	Columnar cell carcinoma of descending colon.	Liver.*	... ...	(ii) Fair nutrition. Hosp. p.m. 45.	(i) 25 Feb. '10. (ii) 26 Feb. '10.
21	A.P. 67/10	F	58	Columnar (becoming transitional) cell carcinoma of sigmoid flexure.	Liver, ovary, meso-colic glands.	Old pulmonary tubercle.	(ii) Moderate nutrition.	(i) 20 Dec. '09. (ii) 16 March '10. (iii) Left inguinal colotomy.
22	J.S. 53/10	M	70	Columnar cell carcinoma of sigmoid flexure.	None.	Peritonitis.	(ii) Well nourished. Hosp. p.m. 44.	(i) 18 Feb. '10. (ii) 25 Feb. '10. (iii) Left inguinal colotomy.
23	E.C. 103/10	F	70	Columnar cell carcinoma of sigmoid flexure.	None.	... ...	(ii) Emaciated.	(i) 21 Oct. '09. (ii) 9 May '10. (iii) Exploratory Laparotomy. Left splenic colotomy.
24	M.Y. 101/10	F	29	Columnar cell carcinoma of rectum.	None.	Chronic peritonitis. Consecutive nephritis.	(ii) Much wasting.	(i) 2 Feb. '10. (ii) 5 May '10.
25	M.W. 74/10	F	39	Columnar cell carcinoma of rectum.	None.	Perforation. Peritonitis.	(ii) Body thin. Hosp. p.m. 64.	(i) 22 March '10. (ii) 23 March '10.
26	K.B. 23/10	F	58	Columnar cell carcinoma of rectum.	Liver, ovary; iliac, lumbar, and inguinal glands.	Œdema of legs. Right pleural effusion. Ascites.	(ii) Moderately nourished.	(i) 18 Oct. '09. (ii) 23 Jan. '10.

27	C.F. 259/10	F	59	Columnar cell carcinoma of gall-bladder.	Liver.	Gallstones.	(ii) Body stout. Hosp. p.m. 203.	(i) 12 Oct. '10. (ii) 30 Oct. '10.
28	M.C. 293/10	F	67	Columnar cell carcinoma of gall-bladder.	Liver, adrenals, lung, portal glands.	Gallstones.	(ii) Thin. Hosp. p.m. 237.	(i) 12 Dec. '10. (ii) 17 Dec. '10.
29	W.C. 80/10	M	69	Columnar cell carcinoma of gall-bladder.	None.	Gallstones.	... ... Hosp. p.m. 63.	(i) 13 Nov. '09. (ii) 23 March '10.
30	R.H. 58/10	F	56	Columnar cell carcinoma of head of pancreas.	Liver; portal lumbar, iliac, and bronchial glands.	... ...	(ii) Fairly nourished.	(i) 25 Jan. '10. (ii) 2 March '10.
31	D.C. 278/10	M	62	Spheroidal cell carcinoma of pancreas.	Liver	... ...	(ii) Fair nutrition. Hosp. p.m. 222.	(i) 6 Nov. '10. (ii) 2 Dec. '10.
32	M.T. 115/10	F	62	Malignant hypernephroma left kidney.	Liver.	... ...	(ii) Emaciated.	(i) 9 Feb. '10. (ii) 28 May '10.
33	J.H. 223/10	M	72	Squamous cell carcinoma of urinary bladder.	Liver, spleen, left adrenal.	... ...	(ii) Well nourished.	(i) 17 Aug. '10. (ii) 1 Oct. '10. (iii) Supra-pubic cystotomy.
34	R.B. 17/10	F	42	Spheroidal cell (colloid) carcinoma of ovary.	Liver, lung, peritoneum.*	... ...	(ii) Moderate nutrition. Hosp. p.m. 38.	(i) 8 Feb. '10. (ii) 17 Feb. '10.
35	C.F. 118/10	F	45	Papilliferous cystadenoma left ovary.	Appendix vermiformis, kidneys, peritoneum; lumbar, sacral, and mesenteric glands.	Chronic peritonitis. Left pyonephrosis.	(ii) Emaciated.	(i) 8 April '10. (ii) 1 June '10.
36	M.G. 288/10	F	47	Columnar cell carcinoma of ovary.	Liver, pleura, peritoneum, lumbar glands.	... ...	(ii) Greatly emaciated.	(i) 3 Oct. '10. (ii) 12 Dec. '10.
37	F.P. 37/10	F	53	Columnar cell carcinoma of ovary.	Liver, skin.	... ...	(ii) Great emaciation.	(i) 19 June '09. (ii) 11 Feb. '10. (iii) Left inguinal colotomy.

TABLE III.—SYNOPSES OF POST-MORTEM CASES—*cont.*

No.	Initials and Cancer Register Number.	Sex.	Age at Death.	Nature of new growth and part primarily affected.	Sites of Secondary new growth.	Other morbid changes present.	(i.) Congenital abnormalities. (ii.) General remarks.	(i.) Date of admission. (ii.) Date of death. (iii.) Surgical operation, if any.
38	E.J. 242/10	F	53	Villous columnar cell carcinoma of left ovary.	Right ovary, liver, pancreas, lung, mesenteric glands.	Double hydronephrosis.	(ii) Emaciated.	(i) 9 Aug. '10. (ii) 22 Oct. '10.
39	K.W. 132/10	F	55	Cystadenoma of ovaries.	None.	... ...	(ii) Great emaciation.	(i) 18 March '10. (ii) 16 June '10.
40	M.A.J. 249/10	F	62	Papilliferous cystadenoma of ovary.	Ovary, peritoneum, pleuræ, stomach, dura mater, lumbar and mesenteric glands.	Double pleural effusion.	(ii) Well nourished.	(i) 3 Dec. '09. (ii) 27 Oct. '10.
41	E.S. 79/10	F	46	Columnar (becoming spheroidal) cell carcinoma of corpus uteri.	None.	Double hydronephrosis.	(ii) Emaciated.	(i) 15 March '10. (ii) 1 April '10.
42	M.A.R. 63/10	F	55	Columnar cell carcinoma of corpus uteri.	Lumbar glands.	... ...	(ii) Fairly nourished.	(i) 9 Feb. '10. (ii) 10 March '10.
43	H.D. 273/10	F	64	Columnar cell carcinoma of corpus uteri.	Right ovary, liver, kidney, aorta, lumbar glands.	... ...	(ii) Fair nutrition.	(i) 8 Nov. '10. (ii) 1 Dec. '10.
44	K.J. 105/10	F	33	Squamous cell carcinoma of cervix uteri.	Lung, peritoneum, lumbar,* aortic,* and mesenteric glands.	... ...	(ii) Emaciated.	(i) 2 April '10. (ii) 12 May '10.
45	E.B. 26/10	F	35	Squamous cell carcinoma of cervix uteri.	Lumbar and iliac glands.	... ...	(ii) Fairly nourished.	(i) 5 Nov. '09. (ii) 28 Jan. '10.

46	E.S. 260/10	F	12	Squamous cell carcinoma of cervix uteri. Double papilliferous ovarian cystadenoma.	None.	... ...	(ii) Well nourished.	(i) 28 Dec. '09. (ii) 6 Nov. '10.
47	B.C. 209/10	F	51	Squamous cell carcinoma of cervix uteri.	None.	Double hydronephrosis.	(ii) Emaciated.	(i) 8 Jan. '10. (ii) 12 Sept. '10.
48	A.E. 298/10	F	52	Squamous cell carcinoma of cervix uteri.	Ovaries, peritoneum.	Gallstones.	(ii) Well nourished.	(i) 25 Nov. '10. (ii) 18 Dec. '10
49	E.J.D. 287/10	F	53	Squamous cell carcinoma of cervix uteri.	None.	Double hydronephrosis.	(i) Patent foramen ovale. (ii) Well nourished.	(i) 14 April '10. (ii) 11 Dec. '10.
50	M.E.M. 61/10	F	61	Squamous cell carcinoma of cervix uteri.	Liver.	Recto-vaginal fistula.	(ii) Some emaciation.	(i) 18 Sept. '09. (ii) 11 March '10.
51	E.E. 271/10	F	63	Squamous cell carcinoma of cervix uteri.	Ovary, sigmoid.	Double hydronephrosis.	(ii) Well nourished.	(i) 9 Nov. '10. (ii) 23 Nov. '10.
52	S.A.W. 183/10	F	63	Squamous cell carcinoma of cervix uteri.	Lumbar glands.	Gallstones.	(ii) Emaciated.	(i) 15 June '10. (ii) 17 Aug. '10.
53	M.A.B. 8/10	F	67	Squamous cell carcinoma of cervix uteri.	Lumbar glands.	... ...	(ii) Great emaciation.	(i) 26 Nov. '09. (ii) 13 Jan. '10.
54	K.J. 244/10	F	34	Squamous cell carcinoma of vulva.	Inguinal glands.	Single "pure cholesterin" gallstone.	(ii) Extremely stout.	(i) 28 Oct. '09. (ii) 25 Oct. '10.
55	J.E. 303/10	F	60	Squamous cell carcinoma of vulva.	Inguinal glands.	... ...	(ii) Extreme emaciation.	(i) 5 July '10. (ii) 30 Dec. '10.
56	F.E.D. 224/10	F	42	Spheroidal cell carcinoma of left breast.	Right breast, liver, pericardium, pleura, peritoneum; axillary, bronchial, lumbar, and portal glands.	Ascites.	(ii) Well nourished.	(i) 4 Aug. '10. (ii) 3 Oct. '10.

TABLE III.—SYNOPSES OF POST-MORTEM CASES—*cont.*

No.	Initials and Cancer Register Number.	Sex.	Age at Death.	Nature of new growth and part primarily affected.	Sites of Secondary new growth.	Other morbid changes present.	(i.) Congenital abnormalities. (ii.) General remarks.	(i.) Date of admission. (ii.) Date of death. (iii.) Surgical operation, if any.
57	M.H. 239/10	F	48	Spheroidal cell carcinoma of right breast.	Liver, left adrenal, pleura, rib, vertebræ, skin, humerus; axillary, bronchial, and lumbar glands.	... ...	(ii) Some emaciation.	(i) 30 May '10. (ii) 15 Oct. '10. (iii) Amputation of breast.
58	A.B. 142/10	F	48	Spheroidal cell carcinoma of right breast.	Thyroid, lung, liver, adrenal; supraclavicular, mesenteric, and lumbar glands.	Right pleural effusion.	(ii) Emaciated.	(i) 17 May '10. (ii) 3 July '10. (iii) Amputation of breast.
59	M.I. 143/10	F	53	Spheroidal cell carcinoma of right breast.	Liver, lung, adrenal; mesenteric, axillary, and lumbar glands.	... ...	(ii) Some emaciation.	(i) 17 March '10. (ii) 2 July '10. (iii) Amputation of breast.
60	C.W. 275/10	F	54	Spheroidal cell carcinoma of right breast.	Lung, adrenals, mesenteric glands.	Double pleural effusion.	(ii) Very emaciated.	(i) 2 July '10. (ii) 5 Dec. '10.
61	C.S. 146/10	F	55	Spheroidal cell carcinoma of left breast.	R. breast, lung, liver, adrenal, lymphatic glands.	... ...	(ii) Emaciated.	(i) 4 June '10. (ii) 5 July '10.
62	M.H. 296/10	F	59	Spheroidal cell carcinoma of right breast.	Lung, pleura, pericardium, vertebræ; bronchial and mesenteric glands.	... ...	(ii) Emaciated.	(i) 2 Nov. '10. (ii) 16 Dec. '10.
63	D.P. 110/10	F	60	Spheroidal cell carcinoma of breast.*	Liver.	... ...	(ii) Some emaciation.	(i) 6 Jan. '10. (ii) 26 May '10. (iii) Amputation of breast.

64	J.S. 56/10	F	62	Spheroidal cell carcinoma of right breast.	Vertebræ, axillary glands, stomach.	... ...	(ii) Fair nutrition.	(i) 3 March '09. (ii) 28 Feb. '10.
65	W.S. 157/10	F	63	Spheroidal cell carcinoma of right breast.	Left breast, heart, lung, intestine, pancreas, peritoneum, uterus, broad ligament, Fallopian tube.	Right pleural effusion.	(ii) Body stout.	(i) 21 June '10. (ii) 24 July '10.
66	A.L. 83/10	F	64	Spheroidal cell carcinoma of left breast.	Sternum, pericardium, bronchial glands.	Œdema of left arm.	(ii) Slight emaciation.	(i) 12 March '10. (ii) 11 April '10.
67	S.O. 20/10	F	65	Spheroidal cell carcinoma of left breast.	Lung, liver, skull, dara mater, pleura; axillary, cervical, and bronchial glands.	... ...	(ii) Well nourished.	(i) 7 Dec. '09. (ii) 21 Jan. '10.
68	E.W. 99/10	F	67	Spheroidal cell (colloid) carcinoma of left breast.	Lung, sternum, axillary glands, skin.	Gallstone.	(ii) Very fat.	(i) 9 March '09. (ii) 2 May '10.
69	E.B. 55/10	F	87	Spheroidal cell carcinoma of right breast.	Skin, pleuræ; portal, mediastinal, and axillary glands.	Œdema of right arm. Double pleural effusion. Gallstones.	(i) Accessory spleen. (ii) Fairly nourished.	(i) 1 May '08. (ii) 28 Feb. '10.

SARCOMA AND CARCINOMA.

1	A.H. 196/10	F	64	Squamous cell carcinoma of cervix uteri, with sarcomatous change of stroma.	None.	Left hydronephrosis. Thrombosis of common iliac veins.	(ii) Emaciated.	(i) 20 Sept. '09. (ii) 28 Aug. '10.

TABLE III.—SYNOPSES OF POST-MORTEM CASES—*cont.*

No.	Initials and Cancer Register Number.	Sex.	Age at Death.	Nature of new growth and part primarily affected.	Sites of Secondary new growth.	Other morbid changes present.	(i.) Congenital abnormalities. (ii.) General remarks.	(i.) Date of admission. (ii.) Date of death. (iii.) Surgical operation, if any.
					SARCOMATA.			
1	W.H.L. 126/10	M	45	Mixed cell sarcoma of neck.	Brain, meninges.	Purulent meningitis.	(ii) Great emaciation.	(i) 20 Oct. '08. (ii) 12 June '10.
2	J.C. 241/10	F	57	Mixed cell sarcoma of corpus uteri.	None.	Right hydronephrosis.	(ii) Well nourished.	(i) 5 Oct. '10. (ii) 20 Oct. '10.
3	N.M. 66/10	F	20	Small round cell sarcoma of groin.	Pancreas, duodenum, gall-bladder, thyroid, ovary. Inguinal, iliac, lumbar, mesenteric, cervical, and portal glands.	Jaundice. Œdema of legs.	(ii) Well nourished.	(i) 23 Oct. '09. (ii) 15 March '10.
4	R.A. 33/10	M	26	Small round cell sarcoma of lung.	None.	... ...	(ii) Fair nutrition. Hosp. p.m. 27.	(i) 10 Jan. '10. (ii) 8 Feb. 10.
5	E.W. 262/10	F	47	Small round cell sarcoma of urinary bladder.	Uterus, broad ligament.	Double hydronephrosis.	(ii) Well nourished.	(i) 17 Oct. '10. (ii) 8 Nov. '10.
6	W.M. 36/10	F	3½	Chloroma.	Bones generally †	... ...	(ii) Well nourished. Hosp. p.m. 29.	(i) 3 July '09. (ii) 10 Feb. '10.

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† Case recorded in IXth Cancer Report, 1910.

ENDOTHELIOMATA.

1	E.M. 272/10	F	32	Endothelioma of pleura.	Liver, pancreas.	... ...	(ii) Well nourished. Hosp. p.m. 219.	(i) 10 Oct. '10. (ii) 30 Nov. '10.
2	E.J.W. 131/10	F	40	Endothelioma of cervix uteri.	Ileum, ovaries, lumbar glands, lumbar vertebræ.	Double hydronephrosis.	(ii) Emaciated.	(i) 18 March '10. (ii) 16 June '10.
3	H.S.C. 57/10	F	49	Endothelioma of cervix uteri.	Liver, urinary bladder. Inguinal, mesenteric, and posterior mediastinal glands.	General peritonitis.	(ii) Some emaciation.	(i) 3 Oct. '08. (ii) 2 March '10.
4	L.M. 119/10	F	56	Endothelioma of cervix uteri.	Liver, omentum, iliac and lumbar glands.	Right hydronephrosis. Left pyonephrosis.	(ii) Very emaciated.	(i) 18 Jan. '10. (i) 1 June '10. (iii) Hysterectomy.
5	L.O. 251/10	F	57	Endothelioma of left superior maxilla.	Cheek, lungs, adrenals, liver, cervical glands.	Left pleural effusion.	(ii) Emaciated.	(i) 5 May '10. (ii) 27 Oct. '10.
6	M.H. 254/10	F	62	Endothelioma of right breast.	Lung, diaphragm, rib, femur.	Right pleural effusion. Gallstones. Spontaneous fracture of femur.	(ii) Well nourished.	(i) 3 March '10. (ii) 1 Nov. '10. (iii) Amputation of breast.
7	M.H. 300/10	F	80	Endothelioma of right breast.	Axillary glands.	... ...	(ii) Well nourished.	(i) 31 Oct. '10. (ii) 28 Dec. '10.

HISTOLOGY DOUBTFUL.

1	H.W. 125/10	M	52	Malignant disease of urinary bladder (nature doubtful).	None.	Jaundice. Gastric and duodenal ulcers. Hydronephrosis.	(ii) Well nourished.	(i) 19 April '10. (ii) 12 June '10.

TABLE III.—SYNOPSES OF POST-MORTEM CASES—*cont.*

No	Initials and Cancer Register Number.	Sex.	Age at Death.	Nature of new growth and part primarily affected.	Sites of Secondary new growth.	Other morbid changes present.	(i.) Congenital abnormalities. (ii.) General remarks.	(i.) Date of admission. (ii.) Date of death. (iii.) Surgical operation, if any.
2	E.T. 236/10	F	53	Primary malignant ‡ disease (? mixed cell sarcoma, or squamous cell carcinoma) of vulva. Primary columnar cell carcinoma of sigmoid flexure.	Lumbar glands (secondary to vulva).	... ...	(ii) Well nourished.	(i) 2 June '10. (ii) 14 Oct. '10.
3	A.E.H. 14/10	F	55	Malignant disease of cervix uteri (? columnar cell carcinoma).	Liver, iliac and lumbar glands.	Right hydronephrosis.	(ii) Great emaciation.	(i) 29 June '09. (ii) 13 Jan. '10.
4	A.C. 256/10	F	56	Malignant disease of uterus.	Liver, pleura, mediastinal and mesenteric glands.	Left hydronephrosis. Right pyonephrosis.	(ii) Very emaciated.	(i) 23 Aug. '10. (ii) 2 Nov. '10. (iii) Hysterectomy.
5	S.C. 274/10	F	60	Malignant disease of corpus uteri.	Femoral glands.	Gallstone.	(ii) Well nourished.	(i) 2 Dec. '10. (ii) 4 Dec. '10.
6	J.M. 182/10	M	73	Carcinoma (? type) of urinary bladder.	Liver, humerus,§ abdominal wall.§	... ...	(ii) Well nourished.	(i) 18 June '10. (ii) 17 Aug. '10.

‡ Double primary.
§ These metastases contained bone.

CANCER INVESTIGATION AT THE MIDDLESEX HOSPITAL, 1900-1910: A RETROSPECT.

BY W. S. LAZARUS-BARLOW.

THE present paper is an attempt to summarise the work of the Cancer Research Laboratories of the Middlesex Hospital during the ten years (1900–1910) since their foundation, to gather the most important results published in the ten volumes of Cancer Reports into a more or less connected whole, and to indicate as far as possible the lines upon which research in the laboratories will probably be carried out during the next few years. Such a course is the more reasonable at the present time owing to the fact that the opening of the new laboratories will inaugurate a new era in the history of Research on Cancer at the Middlesex Hospital.

It has not been thought necessary to enter into details in the following pages owing to the publication of a full Index of the Contents of the first ten volumes of Cancer Reports. It is hoped that this Index may be of use to all interested in the subject of cancer, for the number of questions that have been investigated with more or less fulness is considerable.

Ten years ago but little was known concerning the cancerous process beyond its general clinical history, the microscopical characters of the varieties of growth concerned, and its incurability by any therapeutic means. Isolated observations had shown that a similar or identical process affects lower animals, and its transmissibility by subcutaneous inoculation in the case of mice had been proved. It was known that in very rare instances the disease might undergo spontaneous cure, and the remarkable results which had followed recognition of the fact that micro-organisms are the cause of many diseases had led to a suspicion that cancer also might depend upon a parasite. Whether that parasite were animal or vegetable was a subject of discussion, and while most supporters of the parasitic view held that the cause was a vegetable micro-organism, there was the greatest diversity of opinion upon the actual variety of vegetable organism concerned.

It was at this time that the subject of cancer forced itself anew upon the attention of the authorities of the Middlesex Hospital and led to the formation of the Cancer Research Laboratories of that institution. This was followed a little later by the establishment of the Imperial Cancer Research Fund in England, and, subsequently, of the Central Committee for Cancer Research in Germany, and since then by the establishment of a committee for Cancer Research in almost all the countries of the civilised world. It is but few of these associations that possess laboratories for definite research in cancer so that their investigations must be prosecuted in special directions, while in the case of those laboratories specially devoted to Cancer Research the magnitude of the subject has led to some degree of specialisation.

At the Middlesex Hospital, with its large amount of cancer material and with its records of cases reaching back to 1746, it is natural that the researches should have been primarily devoted to consideration of the disease as it affects man, but the existence within the confines of the hospital of a definite research laboratory has enabled the workers to utilise that human cancerous material to the fullest extent by means of a number of other investigations in other directions that would have been impossible without a laboratory. It is to be understood, therefore, that the present paper is not a retrospect of Cancer Research in general during the past ten years, but only of that portion of the whole which has been carried out at the Middlesex Hospital. I have to indicate what contribution the laboratories of the Middlesex Hospital have made to our knowledge of cancer, and how the problems afforded by that most problematical disease have been answered or partially answered or modified by that work. When this has been done it will be possible to forecast in some degree the direction that should be taken by our investigations during the next few years.

Investigations mainly of a Statistical Character.

The investigations to which reference is made in the present section are based on the records of about 8,000 cases of cancer, about 250,000 cases of non-malignant disease, and about 13,000 autopsies, which have occurred at the Middlesex Hospital since 1853. The questions it has been sought to answer by means of these records are of the following kind. Is cancer on the increase? Is cancer a hereditary disease? With what frequency does cancer affect the various regions of the body? How does the clinical picture vary with difference in the primary seat of the disease? What is the frequency with which metastases occur? How does the situation of the primary disease influence the subsequent duration of life and the number and disposition of metastases? What degree of amelioration or of prolongation of life can the various operations bring about? What is the age incidence of cancer affecting various primary sites? Is the incidence of cancer the same in all climates?

Of these questions some have been specifically dealt with in separate papers, others can be answered by comparison of data afforded in various short monographs on cancer at various sites, and for these the General Index becomes necessary. Papers of the latter class do not lend themselves to examination here, though reference will be made below to the scheme upon which they have been compiled. Concerning the questions of a possible increase in cancer, of heredity, and of age incidence, something may be said.

Is Cancer on the increase?—An attempt was made to arrive at the answer to this question by enquiring into the experience of the Middlesex Hospital and of St. George's Hospital as to the ratio borne by deaths from cancer to deaths from non-cancerous diseases over a period of years. First, then, in order to eliminate errors due to improvement in education of the patients, improvements in diagnosis, improved means of transit, &c., the cases were placed in categories according to the year in which they attained the age of thirty-five years. All the diagnoses were confirmed by autopsy in the case of malignant disease, and the large majority in the case of non-malignant disease. Having dealt with the complete post-mortem records of the hospital concerned, data of the following kind were obtained, males and females being considered separately.

Of M	healthy males aged 35 in year	A, X	subsequently died from malignant	Y	from non-malignant disease.
Of N	Ditto	B, X′	Ditto	Y′	Ditto
Of O	Ditto	C, X″	Ditto	Y″	Ditto

and so on.

In this way a series of ratios was obtained which showed, when plotted out on a curve, that the experience of the Middlesex Hospital was that cancer had been increasing steadily since the beginning of the nineteenth century in males and was still increasing, but that in females it increased up to about the year 1874, and since that time had maintained its high level unaltered. Though differing in details the records of St. George's Hospital yielded similar evidence.

This enquiry was next directed to ascertain whether this increase of cancer affected all the primary sites of the disease or was particularly associated with certain of them, and it was concluded that cancer at all the primary sites had increased, but that a greater increase had taken place in cancer affecting the alimentary tract than elsewhere.

Is Cancer Hereditary?—The data for this enquiry were obtained from the family histories of the patients by members of the Laboratory Staff, and were independently considered by Professor Karl Pearson, F.R.S. It was concluded that the 3,000 cases of cancer dealt with afforded no evidence that the disease is hereditary.

The Age Incidence of Cancer affecting various Sites.—The chief value of this paper lies in the tables included therein. The cases are divided into males and females, into quinquennial age-periods, and each of the following sites is dealt with separately, viz., lip, tongue, other parts of mouth, œsophagus, stomach, colon, rectum, anus (constituting the alimentary tract); liver and pancreas (constituting glands accessory to the alimentary tract); penis, scrotum, prostate, uterus, vagina, vulva (constituting the generative tract); bladder, breast, skin, rodent cancer, and larynx (constituting "other sites"). It appears from the paper that the liability of males to suffer from cancer of the alimentary tract is approximately seven times as great as it is in females, and that the difference in liability of men and of women to suffer from cancer of the alimentary tract gradually and uniformly diminishes as the seat of the growth is lower in that tract. It further appears that, broadly speaking, in both sexes the generative system, including therewith the breast, affords the early cases of cancer, that the cutaneous system affords the late cases of cancer, and that the intermediate group is afforded by the alimentary system. Evidence is brought forward to show that cancer affects women earlier than men, not only by reason of the relatively early age at which the disease affects the uterus and breast in females, but also when organs common to the two sexes are considered and when attention is concentrated on cases of the disease occurring below the age of thirty-five years. Lastly, it was found that in males 80 per cent. of all cancer affected the alimentary tract, and that in females 80 per cent. of all cancer affected the generative tract including therewith the breast.

Statistical Monographs on Cancer at various Sites.—From the records the cases occurring at given sites have been collected, and the data thus obtained have been divided under various headings grouped together as (A) clinical or (B) pathological. The clinical group deals with such points as age, site of appearance of the growth in the organ in question, ulceration, pain, hæmorrhage, antecedent conditions, earliest symptom, subsequent changes, including emaciation, the occurrence of metastases, &c., marriage, fertility, occupation, family history, operations, duration of life, with and without operation. Special points (e.g. the occurrence of jaundice in primary malignant disease of the liver) are considered whenever necessary. The pathological group includes a full description of the situations of metastatic deposits as determined at the autopsy together with histological details. The "primary sites" investigated in this way are as follows: breast, uterus, vagina and vulva, ovary, prostate, thyroid, lip, tongue, œsophagus and upper pharynx, stomach, small intestine and colon, sigmoid and rectum, pancreas, liver and bile passages, peritoneum.

The general reliability of a series of data derived from the records of fifty years (1853–1903) as was used in compiling the above monographs was tested by working out a fresh series derived from the more recent and, generally, more satisfactory records of five years (1904–8) in the cases of cancer of the uterus and cancer of the breast. It was found that the values obtained in the more recent series only differed from those given by the older series in insignificant details. The information given in the above-mentioned monographs may therefore be regarded as giving the experience of the Middlesex Hospital in a substantially correct form.

Cancer and Climate.—The question whether the incidence of cancer is the same in cold and warm as in temperate climates is unavoidably mixed at the present time with the question of cancer and race. The latter subject has not been dealt with in the Middlesex Hospital laboratories, but a comparison has been drawn between the incidence of the disease in Iceland, in Lahore, and at the Middlesex Hospital. Too much stress must not be laid on the results obtained, for the registration, means for forming diagnoses, &c., vary considerably at the three places. It appears, however, that at Lahore cancer of the male generative organs and skin and lower alimentary tract, including primary disease of the liver, are of outstanding frequency, whereas at the Middlesex Hospital the seats mentioned are but rarely affected with primary cancer. On the other hand, uterine and mammary cancer appear to be much rarer in Iceland than in England or even in Lahore, while cancer of the stomach, which is very infrequent in Lahore, and at the Middlesex Hospital has been far less frequently admitted than cancer of the uterus or breast and somewhat less frequently than cancer of the rectum or lip, is the commonest seat of the disease in Iceland. So also in Lahore it is the lower part of the alimentary tract which is more commonly affected by cancer; at the Middlesex Hospital it has been the upper part. A further difference lies in the relative frequency of sarcoma at Lahore; it appears to be about six times as common, relatively to carcinoma, as, from the experience of the

Middlesex Hospital, it is in England. It is clear, therefore, that statements as to the incidence of cancer can only be applied with justice to the region from which the material for the statistical enquiry was derived.

An interesting point, if it be confirmed by subsequent investigations, is that the Lahore statistics showed Eurasians to be far less frequently the subject of cancer than either Europeans or Indians resident in the same region.

Gallstones and Cancer.—An examination of the post-mortem records of the hospital (1854–1904) on the question of a relation between gallstone formation and cancer showed that the liability of the two sexes to gallstones differs in the same way as their liability to cancer. Thus it was found that about twice as many women as men had died of cancer and about twice as many women as men had been the subject of gallstone. Not all the subjects of gallstone, however, were also subjects of cancer, and sub-dividing the gallstone cases into malignant and non-malignant series, it was found that gallstones are met with in both sexes approximately two and a half times as frequently in carcinomatous as in non-carcinomatous subjects. The liability of gallstones to be associated with carcinoma was apparently independent of the seat of the primary growth when the question was looked at on broad lines, but the matter is somewhat different when primary carcinoma of the gall-bladder and bile passages are considered. Thus gallstones were found post mortem in twenty-seven of thirty-one cases of primary malignant disease of the gall-bladder and bile passages. In the remaining four of the series it is specifically stated that no gallstones were discovered, but no mention is made on the point of pigmentary débris.

Menstruation, Childbirth, and Cancer.—The object of this research was to determine if possible a relation between sexual life and the occurrence of cancer in the female. It was found that the duration of sexual life, i.e., the period between onset of menstruation and menopause, is longest in uterine cancer, shortest in rectal cancer, and that it is intermediate in mammary cancer. But in spite of the fact that sexual life is longest in cases of uterine cancer, the average childbearing period, i.e., from birth of first to birth of last child, is shorter and the number of pregnancies is fewer than in cancer at other sites.

This short review of the statistical papers that have been compiled by the workers in the Cancer Research Laboratories of the Middlesex Hospital indicates that a very large number of "values" has been obtained. Their chief use at present is for comparison with results obtained by other workers with other material. At some later stage in our knowledge the facts which these "values" disclose will become explained, and even now some of them indicate lines of research. In the majority of cases, however, statistics can only raise a question. They can tell us, for example, that emaciation has occurred at the Middlesex Hospital in 81 per cent. of uterine cases, and in only 46 per cent. of mammary cases. The information is useful from a clinical point of view and as indicating a subject for research, but it offers no suggestion as to the lines upon which that subject should be worked out. Hence these statistical enquiries, with the immense amount of labour which they have entailed, are to be regarded as a framework upon which the more strictly scientific investigations must be built.

Investigations mainly of a Bacteriological Character.

Only three researches of an essentially bacteriological character have been carried out in the laboratory. Two of these dealt with the bacterial flora of carcinoma and sarcoma and with the pathogenetic action of blastomycetes, respectively, and were undertaken at a time when Sanfelice, Roncali, Pernice, Busse, and others were asserting that this variety of micro-organism is the cause of cancer; the third, on the presence of micrococci in tumours, was undertaken when Doyen insisted that his so-called *Micrococcus neoformans* was the ætiological factor. In the case of the blastomycetes it was shown that when cultures of carcinomatous and sarcomatous material were made under stringent conditions to avoid aerial contamination, no blastomycetes were found, and on the other hand that inoculation into animals of cultures of known blastomycetes was followed in successful cases by the production of granulomatous masses, and not by the production of carcinoma or even sarcoma. In the case of the micrococci it was found that a micro-organism hardly distinguishable from *Staphylococcus albus* occurs as an inhabitant of practically all malignant tumours and of some simple tumours as well. A long series of inoculation experiments (unpublished) failed to give evidence that the micro-organism in question possesses pathogenetic powers.

Investigations mainly of a Histological Character.

The investigations dealt with under the above heading are very diverse. In all instances the microscope has been the chief apparatus used, and in some the researches are self-contained, but in most cases the research has presented the histological aspect of a question having wider bearings and needs correlation with other researches of a physical, a chemical, or a therapeutic kind. Reference to some of these researches of correlation will be made later, but in many instances the allied researches are still in progress. For convenience of description they may be divided into the four following classes: investigations on the histological characters of new growths; investigations into the mode of spread of malignant new growths; investigations into the condition of the blood cells in malignant disease; investigations upon precancerous conditions.

Investigations on the Histological Characters of New Growths.—Of this group some papers have been definitely anatomical; others have dealt with the neoplastic cell in respect of its division, its metabolism or its phylogeny.

Anatomical.—In a paper on giant cells in sarcoma the conclusions are drawn (1) that true giant cells are only found in sarcomata arising in connection with bone, while multinucleated cells (to be distinguished from giant cells) are never found in tumours arising in connection with bone; (2) that true myeloid sarcomata occasionally form metastases and then show giant cells in the secondary nodules. From this it follows that if true giant cells be found in a growth it is very unlikely to form metastases, though not impossible, while the presence of multi-nucleated cells must not be regarded as warranting a favourable prognosis.

In another paper the behaviour of elastic fibres in tissues the seat of new growth has been shown to be entirely passive, the fibrils being swept before the advancing neoplasm and no elastic tissue being present therein except those remnants belonging to the pre-existing organ and possibly some of new formation in the blood vessels of the tumour.

A paper that may be included in this group refers to the relation of rodent cancer to squamous-cell carcinoma, and the author compares the clinical history of squamous-cell carcinoma affecting muco-cutaneous surfaces, and the same type of growth affecting cutaneous surface proper. From the resemblance of the clinical history of rodent cancer with that of squamous-cell carcinoma affecting cutaneous surfaces proper, and from the fact that the same growth may show a transition from definitely squamous to definitely rodent growth, and conversely, it is concluded that they are identical in essence. The different appearances of rodent cancer and of squamous-cell carcinoma of cutaneous surfaces would thus be expressions of nothing more than variations in rate of growth.

Mention may here be made of papers describing rare conditions (viz., chondrifying carcinoma, endothelioma of the tongue, ossifying sarcoma of the breast, invasion of an ossifying fibro-myoma by carcinoma, chloroma), or of a comparative kind (viz., mammary tumours in the bitch, tumours in mice). Further reference to them is not necessary here.

Under the anatomical heading may also be mentioned researches upon the modes of division of cancerous and other cells. Eight years ago it was suggested that the cells of malignant neoplasms divide in a manner similar to that obtaining in reproductive cells and unlike that occurring in cells of the body generally. The subject was investigated in these laboratories and it was shown that gametoid division also occurs in the "semi-malignant" papilliferous cystadenoma of the ovary and in the infective but non-malignant gonorrhœal wart. It was therefore concluded that gametoid mitosis is rather symptomatic of a condition of independent existence on the part of the cell than a causative factor in the production of the neoplasm.

Researches mainly of a histological character but having a phylogenetic bearing are found in papers on chorionepithelioma of congenital origin and on endothelioma respectively. The former of these brings forward evidence to show that certain tumours found apart from pregnancy and yet having histological characters closely resembling those of tumours the result of conception, are due to inclusion of cells at a very early date in fœtal development (blastomere, layers of blastocyst or specialised derivatives of layers of blastocyst). The latter series of papers suggests that about 10 per cent. of the growths of mamma and of cervix, hitherto described as spheroidal-cell carcinoma and squamous-cell carcinoma respectively, belong to one single class which is better explained on the view that the growths in question are endotheliomata and therefore mesoblastic than on the view that they are carcinomata and therefore epiblastic.

Investigations mainly of a histological character but bearing upon the metabolism of the cell are represented by a series of papers upon Altmann's granules and their relations to new growths. Altmann's granules are fuchsinophil bodies varying in size from dust-like particles to granules the size of those found in an eosinophil cell. All varieties of cell in the body from the earliest fœtal life (unincubated chick), show the presence of these cells, but in post-natal life they are absent from unstriped muscle, squamous epithelium, the cells of the collecting tubules of the kidney, and the ovum, and from these cells alone. On the other hand they are absent or practically absent from the cells composing a malignant new growth, even though the ancestral cells of that new growth contained Altmann's granules. Thus columnar epithelium of stomach, intestine, or uterus contains numerous Altmann's granules in the cytoplasm of its cells, but the cells of a columnar-cell carcinoma in these regions are devoid of Altmann's granules. So too the cells of a sarcoma are devoid of granules, though the mesoblastic cells of the tissue from which that sarcoma originated contain numerous granules. The absence of granules is characteristic of malignancy, so that a non-malignant epiblastic or mesoblastic new growth or a mass of inflammatory tissue shows cells all of which have a cytoplasm richly provided with Altmann's granules.

The observations upon which the above statements are based are very numerous, and it is not to be supposed that no exceptions have been found. Exceptions to the rule have, however, been infrequent and are chiefly confined to ovarian tumours. It must be noted that absence of Altmann's granules from the cells of a squamous epithelial growth is characteristic whether that growth be malignant or innocent, since the squamous epithelial cell is normally devoid of granules. Cancerous tumours of mice do not yield quite as conclusive evidence as those of man, about one-third of the tumours described as carcinoma or sarcoma in the mouse showing many Altmann's granules in the cytoplasm of their constituent cells.

Investigations of a different but allied kind, and in which the metabolism of the malignant cell itself is not considered, but rather the influence exerted by the malignant cell on neighbouring tissues, were carried out in papers on chondrifying carcinoma and secondary malignant conversion of epithelium respectively. In the case of chondrifying carcinoma, the author concluded that the cartilaginous condition (and in analogous cases the myxomatous condition) of the stroma was brought about owing to an influence exerted by the proliferating carcinoma cells upon the connective tissue stroma in which they lay. Here the malignant cells were in close relation with the stroma cells which they were supposed to influence. In the case of the secondary malignant conversion of epithelium, a duct in the breast, separated through its whole length by unbroken normal connective tissue from the spheroidal-cell carcinoma in its neighbourhood, showed a remarkable proliferation of its lining cells. The masses of cells produced by this proliferation were irregular, and recalled the irregular alveoli of a spheroidal-cell carcinoma, but the individual cells preserved many of the characteristics of cells lining the lactiferous ducts. It was suggested that this change was induced in the duct by the carcinoma in their neighbourhood.

These two researches are of great interest in connection with the recent observations upon the occurrence of sarcoma in mice as the result of transplantation of carcinoma. There is reason to believe that all the stroma of a large mass of mouse cancer, grown after transplantation of a minute portion of a pre-existing case, is wholly provided by the host, and under the conditions in question it is suggested that the host supplies a sarcomatous instead of a normal connective tissue in response to a special stimulus afforded by the transplanted carcinoma cells.

Investigations into the Mode of Spread of Malignant New Growths.—Under this heading are researches upon the spread of carcinoma of the breast, of melanotic sarcoma, and of carcinoma and sarcoma in the female pelvis. They have shown that it is characteristic for mammary carcinoma and melanotic sarcoma to permeate and travel along the lymphatic channels, coincidently inducing changes in the walls of the lymphatics and the tissues immediately surrounding them, whereby the permeating neoplastic cells are deprived of their nourishment. A perilymphatic fibrosis therefore is the response of the organism to the stimulus afforded by the invading carcinoma cells, and brings about a necrosis of those cells. The lymphatic channels concerned are chiefly those of the deep fascia. A modification of the process is seen when carcinoma cells fall into the peritoneal cavity, for then there is reason to believe that the ciliated epithelium of the Fallopian tube sweeps the free cells into its lumen, that they may became lodged in the Fallopian tube, and after perforating the columnar epithelium that they may form definite metastatic nodules in the walls of the tube itself. In the case of mammary carcinoma and melanotic sarcoma, these results have indicated a modification of the ordinary method of operation.

Investigations into the Condition of the Blood Cells in Malignant Disease.—Comparisons have been made between the cells found in the blood in normal individuals, and in those suffering from malignant disease, both on man and on the mouse, while in the latter animal parallel observations have been made upon the bone-marrow. The results of this work show in general that both in mice and man affected with malignant growths there is a slight anaemia, increasing in the later stages of the disease, and that the leucocytes in the blood undergo marked changes. Thus there is both a relative and an absolute great increase in the number of large mononuclear cells, and, in addition, a large absolute increase in polymorphonuclear cells. Examination of the bone-marrow in cancerous mice showed but little variation from the normal, chiefly owing to the increased myeloid activity of the spleen of cancer mice, whereby any altered activity of the bone-marrow is either masked or obviated.

Investigations upon Pre-Cancerous Conditions.—These have been prosecuted in three directions, and partly upon lower animals, though chiefly upon man.

(1) *X-ray Dermatitis.*—In animals histological examination has been made of tissues the seat of X-ray dermatitis artificially produced, while the same subject has been studied in material derived from several human sources. It was shown that the epidermal cells undergo two opposite reactions as the result of the action of X-rays, viz., hyperplasia with the formation of a thickened epithelial layer at certain parts, and atrophy with a thinned layer which ultimately disappears from necrosis at other points. Beneath the atrophic portions in particular, but also beneath the seats of hypertrophy, the cutis shows inflammatory changes with numerous plasma cells in man, but few or none in animals. The epithelial atrophy and necrosis appear to occur at the situation of greatest incidence of the rays, but both atrophy and hypertrophy are usually contiguous.

(2) *Pre-cancerous Conditions in Man.*—Two papers on pre-cancerous conditions in man have given the results of careful examination of very early carcinomata and of conditions such as leucoplakia of vulva and tongue, which are frequently followed by carcinoma. The conditions found were those of a chronic inflammation with large numbers of plasma cells. It is into tissues of this description that the proliferating epithelial cells of a commencing carcinoma insinuate themselves. The author concludes that the tissue cell proliferation occurring round a primary carcinoma is a part of the pre-carcinomatous process, and so far from being regarded as a protective reaction on the part of the tissues must be considered as a preparation for and an assistance to the progress of the growth. It is pointed out that the forma-

tion of metastases is not accompanied by local inflammatory changes of a like kind except in the case of lymphatic glands, and these are rather outlying portions of the prime focus in the pre-cancerous inflammatory conditions by reason of their close connection through lymphatics than separate organs or tissues.

(3) *Posterior Root Ganglia.*—A search was made for possible changes in the posterior root ganglia of man and mice the subjects of carcinoma. It is to be noted that the human and mouse cases are not strictly comparable, since the human cases were spontaneous, whereas the mouse cases were the result of transplantation. In no instance was it possible to conclude that the cancerous condition was reflected in the posterior root ganglia. In the inoculated mice the ganglia were apparently normal, and in man such changes as were found after death on the same side as the new growth were also found in the posterior root ganglia of the other side.

Investigations mainly of a Bio-chemical Character.

Reference will be made in this section to researches upon the composition of the blood and tissues designed with the object of determining whether differences obtain between non-cancerous and cancerous individuals.

Investigation of the specific gravity of the blood and blood plasma showed that in carcinoma a lowering occurs which varies directly with the degree of cachexia; the specific gravity of the blood corpuscles on the other hand does not vary greatly from the normal unless cachexia be extreme. A similar lowering of specific gravity was found to obtain in the cases of the spleen and voluntary muscle, but liver, kidney, and cardiac muscle showed no difference from the normal. It is probable that the changes in spleen and voluntary muscle depend upon alteration in specific gravity of the blood since they also vary directly with the cachexia.

Attempts to discover evidence of the formation of an abnormal protein or a toxin in cancerous material, or in the blood of cancerous individuals, by means of the precipitation test failed completely. Introduction of any of these materials into a rabbit led to the production of an "anti-human" serum alone, and numerous modifications of the experiments failed to show any differences between the sera of rabbits according as they were "immunised" with cancerous or non-cancerous material. They also failed to show that either of these sera acted differently when mixed with the serum of a cancerous or non-cancerous human being, or with the original material used for immunisation, or that human cancerous tissue presented different microscopical appearances when preserved in a rabbit serum prepared by immunisation with human cancerous material than it did when preserved in a rabbit serum prepared by immunisation with human non-cancerous material.

By a spectroscopic method devised in the laboratory estimations were made of the sodium and the potassium contents of the blood and certain tissues in carcinomatous and non-carcinomatous individuals, and also of primary and secondary carcinoma material. It was found that in carcinoma the percentage amount of sodium is but little altered from the normal, whereas there is a considerable increase in the percentage amount of potassium. This is certainly the case with the blood, and (whether it be due to intrinsic differences or to the blood they hold) it is also true of liver, kidney, and spleen. In the case of carcinoma material, though the number of cases examined is small, it appears that the sodium content is about the same in all varieties of carcinoma, and whether the growth be primary or metastatic, while the potassium content on the contrary is greater in squamous than in spheroidal, and is higher in primary than in secondary growths.

Experiments have been conducted upon certain enzymes in carcinomatous and non-carcinomatous cases. Thus ereptic action, autolysis of proteins, and the action of catalase have been compared in reference to the two classes of cases. It was found that when allowance is made for emaciation—a condition which is always associated with a diminution of ereptic, autolytic or catalytic power—no difference obtains between the tissues of cancerous and non-cancerous persons. It is to be noted that these observations do not refer to the cancerous growth itself, which is a subject for future investigation.

In the section devoted to investigations mainly of a histological character reference has been made to Altmann's granules. Attempts have been made to determine the nature of these granules. There is reason to believe that all the granules in cells which stain in the characteristic Altmann manner are not of one and the same kind. Differences in size, in solubility and in stability are recognisable, but so far it has not been found possible to differentiate them in any other ways. Experiment has, however, broadly indicated that the granules belong to a group of proteins having an acid character in reference to the proteins in which they are disposed.

Investigations mainly of a Bio-Physical Character.

In view of the fact that carcinoma is known to have followed more or less closely upon exposure of the skin to the action of X-rays in certain cases, researches have been carried out in the laboratories on the following lines :—Firstly, investigation was made into the question whether non-malignant and carcinomatous tissues show differences in their effects upon a photographic plate in the dark and upon a charged electroscope. Secondly, the effect of exposing living animal cells to the action of X-rays, radium, thorium, or uranium has been studied. And thirdly, investigation has been made into the effect of radium radiations upon some of the chief constituents of the blood, and of the effect of radium emanation upon Altmann's granules.

The inquiry as to the effect of carcinomatous and non-malignant tissues upon a photographic plate in the dark showed that such tissues vary to a considerable degree, but may be placed in groups. Thus lung and spleen dried at 100° C. and powdered have but little effect, whereas liver and kidney lead to a change of the silver in the photographic film, which on development shows itself as a more or less deep punctate silver deposition. Numerous experiments were made to determine the explanation of this silver deposition, and after exclusion of fat, carbohydrate, protein, mineral salts and known extractives as such, it was concluded that in certain instances at least the change depended upon the formation of minute traces of ammonia. A similar deposition of silver is produced by various calculi, vesical, renal and biliary, the greatest effect being the result of allowing a pure cholesterin gallstone to act directly on or at a short distance from the film. In this instance there is reason to believe that the result is not due to the action of ammonia, but it is impossible at present to say what the active agent may be. This "skotographic" action shows certain well-marked differences from the action on a photographic plate of X-rays or radium, in particular it is not exerted through a mica screen. Carcinoma material, primary or secondary, has marked skotographic powers, while a certain degree of effect is exerted by some varieties of micro-organism, notably the pyogenetic cocci *B. tuberculosis* and *B. diphtheriæ*. Most of the other varieties of micro-organism tested were devoid of skotographic power or were very feebly active.

Electrical examination of carcinomatous and non-malignant tissues in dried, fat-extracted powder appeared to show that there is a difference between them, and the carcinomatous tissue gave results that led the author to conclude that it can be separated into residues and extracts which have opposite effects upon electroscopic leak. The matter is highly important, and it will be necessary to extend and repeat the observations under more stringent conditions, as the differences noted are but small, and, though such is believed not to be the case, they may be due to experimental error.

The exposure of animal cells to X-rays, radium, thorium, or uranium has been carried out in the following directions:—1. The ova of *Ascaris megalocephala* have been exposed to X-rays, radium, thorium, and uranium. 2. The action of X-rays and also of radium has been investigated upon silkworms under a great variety of conditions. 3. The action of X-rays on the skin and subjacent tissues of rats and rabbits has been examined microscopically (reference has already been made to this research above). 4. Microscopical examination has been made of secondary carcinomatous skin nodules subjected to the action of pitchblende (radium). From these researches it appears that the radiations derived from these various sources have an undoubted influence upon the growth and metabolism of the animal cell. The influence, however, is not always in one direction. Sometimes a marked acceleration of growth is occasioned; sometimes as marked a retardation with abnormal cell division and ultimate death of the cell. This duality of action is seen in reference to all the varieties of cells mentioned, and frequently opposite actions are recognisable in contiguous cells without, at present, any known difference either in the cells themselves or in the treatment to which individual cells have been subjected.

Investigations into the effect of radium radiations on some of the chief constituents of blood have shown that the *a*-radiation is highly potent. It leads to a leakage of hæmoglobin from the red blood corpuscle, and to disintegration of the leucocyte; it occasions a disappearance of opsonin and of complement from the serum, and from the characters of the curves of disappearance it is clear that opsonin and complement are different substances. Similarly, a disappearance of Altmann's granules from the spleen results from exposure of portions of this organ to radium emanation, and in this instance also there is reason to ascribe the change to the impact of *a* particles.

Researches mainly of a Therapeutic Character.

The last line of research to which attention will be turned is represented in the Reports from the Cancer Laboratories by papers on new therapeutic measures adopted in the cancer wards. Here responsibility, in the first instance, lies with the surgeons in charge of the cases, but assistance has always been rendered whenever necessary by the laboratory staff. Careful examination has been made of the claims put forward on behalf of the Dr. Otto Schmidt serum, the trypsin and amylopsin treatment, violet leaves, betel leaves, molasses, chian turpentine, marigold ointment, cancroin, Doyen's serum, *Micrococcus neoformans* vaccine, Coley's fluid, liquor ammoniæ fortissimus, phenacetin, soamin, atoxyl, thyroid extract, thymus extract, high-frequency current, X-rays, pitchblende.

In the case of X-rays, pitchblende, and radium, though results are discrepant, some cases showing marked improvement; while other apparently similar cases showed marked aggravation, and yet others showed no change, it is felt that research on these lines, linked up with researches of a biophysical kind mentioned in the last section, may profitably be continued. Coley's fluid has met with a partial success in cases of sarcoma, but it is clear that Coley's fluid is almost as variable in its action as X-rays, and certainly its administration has not met with as much success in this country as Dr. Coley claims for it in America. Thyroid extract and thymus extract appeared beneficial in some degree, but hardly enough to warrant a more prolonged trial. The other agents, it was reluctantly concluded, are completely valueless; some of them, indeed, are actually injurious.

It will have been seen that work in the laboratories has been carried out on many different lines during the past ten years. Of these the statistical and histological have received a considerable amount of attention, and it is probable that the former, at all events, has reached a limit. The fact that two statistical investigations (uterus and breast) carried out on the most recent material have shown that similar investigations dealing with a larger amount of material, but collected over a longer period, are substantially correct, is an indication that no benefit would be derived from similar investigations for a number of years to come. The statistical data to be found in the ten volumes of Cancer Reports may be taken as a closely accurate statement of the information which the Middlesex Hospital is able to afford on the points dealt with.

With regard to histological investigations in cancer the matter is somewhat different. There is no doubt that histological researches in cancer, to be of value, must consist in the application of some new method. A limit has been reached in the profitable employment of the ordinary laboratory histological methods to the division and subdivision of groups of neoplasm or to the description and collection of rare varieties; the labour entailed is not justified by the results. But that histological research cannot be discarded is shown by the work that has been done in this laboratory on Altmann's granules. Discovery of the new method which showed that certain granules can be demonstrated by a special method of staining has led to discovery of the fact that there is a practical absence of Altmann's granules in carcinoma and sarcoma, This discovery has not only afforded the first indubitable evidence we have obtained that the cancerous cell differs in its metabolism from its ancestors, not only has opened up an entire research into the nature and functions of these granules, but at the present time affords a means of differentiating between certain conditions hitherto difficult or impossible to differentiate by microscopical examination and a matter of doubt clinically. Determination whether a given mass of redundant tissue is sarcomatous or inflammatory, is columnar-cell carcinoma or endometritis is now possible, and with further extension of our knowledge in this direction a still wider application may be hoped for.

In reference to bio-chemical and bio-physical investigations on the subject of cancer the matter is very different. It may almost be said that we are not even at the present time in a position to attack the heart of the problem. It is clearly unsatisfactory to treat cancer cases with radium or with X-rays until it is known what the effect of these agents is upon the constituents of the cancer or of the healthy tissues or of the blood or other fluids of the body. Research into such questions is, however, justified by the facts that radium and X-rays are known to influence certain cases of cancer profoundly whether for good or for ill, and that they also influence in a similar way the growth of living animal cells of a different kind (ova of Ascaris, silkworm). Similarly it cannot be doubted, since a mass of cancer cells has a different metabolism from that of the normal cells which gave it origin, and shows that different metabolism by a different constitution of its cytoplasm (absence of Altmann's granules), that an enquiry into the characters of this metabolism will be profitable. Nor can it be doubted that the presence of such a mass of cancer cells in the body, with their own altered metabolism and with the degenerative changes they are liable to undergo, must alter the metabolism of the body generally. The occurrence of metastases in some cases and not in others, the whole of that cachexia which plays so large a part in bringing about death, probably also the very fact that a normal epithelium oversteps its proper limits, invades the deeper tissues, and becomes the primary mass of malignant disease must be closely bound up with the bio-chemical changes in the epithelial cells on the one hand and in the tissues generally on the other.

It thus appears that research on bio-chemical and bio-physical lines offers a good probability of yielding profitable results for the ultimate elucidation of cancer as a biological process and, concurrently, for the proper treatment of cancer as a disease. In the absence of some great discovery which of necessity will divert our researches into unexpected directions, one may therefore anticipate that in the Cancer Research Laboratories of the Middlesex Hospital the investigations undertaken during the next few years will proceed largely on the bio-chemical and bio-physical lines which have been indicated by the researches published in the more recent Reports.

AN EXPERIMENTAL INQUIRY INTO THE NATURE OF THE ALTMANN'S GRANULES OF THE KIDNEY.

By HECTOR A. COLWELL and HENRY BECKTON.

In a series of papers * which appeared in previous volumes of these reports, it was shown that Altmann's granules are present in the cells of nearly all normal tissues, the chief exceptions to this rule being unstriped muscle, squamous epithelium, and the pyramidal portion of the kidney. In marked contrast to this almost universal presence in normal cells is their general absence or sparsity in the cells of malignant growths. It follows that the examination of tissues, to determine whether these granules are present, has acquired diagnostic importance in those cases where ordinary histological methods give doubtful results.

It is obvious, however, that, apart from their diagnostic value, study of the granules may afford information regarding the different metabolism of the granular and agranular classes of cell. With that object in view a series of researches has been undertaken in these laboratories, one of which, mainly chemical, forms the subject of the present paper. Details of the histological technique for showing Altmann's granules have already appeared, but it may be said that the method consists essentially in

1. Fixation of the tissue in formol-Müller solution.
2. Staining in aniline-acid-fuchsin.
3. Differentiating in picric acid alcohol, when the granules retain the stain—i.e., are "fuchsinophile," whereas the colour is discharged from the rest of the cell elements of the tissue.

"**Fuchsinophile Reaction.**"—By this term is meant the property which certain substances possess, when stained by a solution of acid fuchsin in aniline water, of retaining the stain upon treatment with picric acid alcohol. The term is essentially a relative one, as by sufficiently long treatment and the employment of large quantities of the reagent the stain can always be discharged. In the case, however, of portions of the same section, or films identically treated upon the same slide, the relative differences in their behaviour on differentiation are easily distinguishable.

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* H. Beckton, "Archives of the Middlesex Hospital," 8th and 9th Cancer Reports; also "Brit. Med. Journ." (1909, 1910), and "Journ. of Path. and Bact." (1909, 1910).

Types of Granule.—At the present stage of our investigations we can recognise, broadly, two types of fuchsinophile granule. In the first class they can be demonstrated when the tissues are fixed in formalin alone, and also in films prepared from scrapings of the fresh tissue, which are merely fixed by slight heat (e.g. 60° C.). Granules of this class are found in the kidney, pancreas, liver, and submaxillary gland.

In the second class, special fixation, as by the formol-Müller method, is essential for their satisfactory demonstration, though *prolonged* fixation in plain Müller's fluid may give results which are nearly as good. Neither formalin alone nor heat is an adequate fixative. Granules of this class occur in lymphocytes. It may be noted that in all cases where granules are capable of fixation, either by slight heat (as in films), or by formalin alone, fixation is better if formol-Müller be employed instead of the other methods. The formol-Müller gives the best fixation of either of the two types of Altmann's granules under consideration.

Distribution of Altmann's Granules in the Kidney.—The kidney presents two distinct zones as regards the presence of granules. In the cortex they are abundant, in the pyramid all but absent. This fact is demonstrable to the naked eye if a section of kidney, cut so as to include both regions, be stained and differentiated as described : the cortex appears bright red, while the pyramidal portion is nearly colourless. It was in consequence of the great number and relatively large size of the cortical granules that the kidney was selected as the organ upon which the present investigations should be conducted. A further point of paramount importance is the fact that in one and the same organ granular and non-granular portions co-exist, comparisons under precisely identical conditions being thus rendered possible. Sheep kidneys were used throughout these experiments, and were obtained directly from the slaughter-house.

Attempts to Isolate the Granules.—Microscopical examination shows that the granules form a large proportion of the cortical tissue. Attempts were therefore made to dissolve them in various reagents, in the hope that the examination of the solutions so obtained might give some clue to their chemical composition. In this we were unsuccessful, for although the fuchsinophile reaction disappeared after prolonged exposure to, e.g. water, no chemical differences were detected between a watery extract of cortex and one of pyramid from the same kidney.

Search was next made for a reagent capable of dissolving the inter-granular non-fuchsinophile elements of the cortex while leaving the granules intact. Small portions of cortex were boiled for a few minutes with 10 per cent. HCl. Fragments removed at short intervals, washed, fixed in formol-Müller, and prepared in the usual way yielded a series of microscopic sections in which preservation of the granule picture was observed while the inter-granular substance showed increasing destruction. Ultimately little more than fuchsinophile granule material remained. The granules, then, were more resistant to hydrochloric acid than the rest of the cell protoplasm. On prolonging the boiling, or increasing the strength of the acid to 20 per cent., the granules disappeared in common with the rest of the tissue. Cortex was next exposed to 50 per cent. HCl in the cold for twenty-four hours. The tissue became so friable that it was readily rubbed into a paste by a glass rod. After thoroughly stirring, the insoluble residue was separated by the centrifuge. On preparing a film, staining, differentiating, and examining microscopically, it was seen to consist of a number of strongly fuchsinophile granules identical in appearance with those found in the cortex before treatment with acid. Taking this in conjunction with the results obtained by boiling portions of cortex with 10 per cent. HCl, we seemed justified in concluding that the residue so obtained consisted to a large extent simply of the granules, though undoubtedly modified by the treatment they had undergone.

The main part of the tissue which went into solution consisted of the non-fuchsinophile inter-granular material, and gave a powerful biuret reaction. It is noteworthy, however, that a film prepared from this solution, after staining and differentiating, gave a marked *fuchsinophile* reaction ; though the fuchsinophile material was disposed not as discrete granules, but as a more or less homogeneously staining layer. Under the action of an acid, therefore, a non-fuchsinophile material became fuchsinophile.

The more ready solubility of the non-granular parts of the kidney in hydrochloric acid is confirmed by the following experiment: Equal weights (4·15 gram) of pyramid and cortex were taken from the same kidney. To each was added 50 c.c. of 50 per cent. HCl, and both were allowed to stand at the room temperature for eighteen hours. Most of the supernatant fluid was pipetted off, and the undissolved residue was in both cases disintegrated by means of a glass rod. On centrifugalising to constant volume in tubes of equal bore, it was seen that the residue from the cortex was much greater in amount than that from the pyramid, thus showing that more of the latter had been dissolved.

This was confirmed by a comparison of the specific gravities of the respective solutions by Roy's method.*

Chemical Examination of the Residue from the 50 per cent. HCl Digestion.—The residue, although resistant to the action of 50 per cent. HCl, is nevertheless slightly soluble in it in the cold, and rapidly dissolves on warming. Solutions so obtained, as well as the residue itself, give a powerful biuret reaction.

The Effect of Hydrolysis.—To determine this, some of the residue from the 50 per cent. HCl. digestion was heated in a flask (fitted with a reflux condenser) with a further amount of 50 per cent. acid for about six hours. The solid matter readily dissolved upon the application of heat, and the liquid assumed a dark-brown colour. At the end of six hours a portion of the liquid was removed and tested by the biuret method. The result was negative, thus showing that the portion had undergone hydrolysis. A film of this hydrolyzed material was next prepared. Some difficulty was experienced in obtaining films which did not wash off when treated with the staining and differentiating reagents; but in those which were satisfactory the fuchsinophile reaction was found to have completely disappeared. The amino-acids then (or rather their hydrochlorides) do not possess this fuchsinophile power. This was further confirmed by staining a film of egg-white (which is non-fuchsinophile) mixed with alanin (α-aminopropionic acid). No fuchsinophile reaction was given.

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* "Journ. Physiol.," "Proc. Physiol. Soc." March, 1884.

The acid solution derived from the cortex-digestion, which, as previously said, was powerfully fuchsinophile, was also set to hydrolyze. On preparing films as before the fuchsinophile reaction was found to have been lost. Consequently of

1. The intergranular portion of the cortex,
2. The acid albumin derived therefrom, and
3. The amino-acids produced by the hydrolysis of 2,

only the acid-albumin phase gives the fuchsinophile reaction.

In a further experiment the pyramid was carefully removed from a sheep's kidney, and examination of a control film showed it to be non-fuchsinophile. The pyramid was next transferred to 50 per cent. HCl for twelve hours. A considerable portion was dissolved, but sufficient residue was left for films to be made; these appeared strongly fuchsinophile.

In the following experiment films were made with scrapings from cortex and pyramid respectively. They were dried and fixed by gentle heat, then treated with 50 per cent. HCl for different times, stained for three minutes in aniline acid-fuchsin, and differentiated in picric alcohol for ten minutes. The results were as follows:—

Time of Exposure to 50 per cent. HCl.	Cortex.	Pyramid.
Control	Granules well marked and discrete.	No granules.
Six minutes	Granules well marked, but slight general fuchsinophile reaction.	No granules. Patchy fuchsinophile reaction.
Four hours	Granules as before. Fuchsinophile reaction generally well marked.	No granules. Fuchsinophile reaction general.

The experiments in the foregoing paragraphs indicate that the fuchsinophile reaction depends upon the presence of an acid albumin. To test the effect of alkali, the following experiment was performed :—The pyramid of a kidney was treated with 50 per cent. HCl for eighteen hours. The solution was, of course, strongly fuchsinophile. On rendering this distinctly alkaline with NaOH, the fuchsinophile reaction disappeared, but on re-acidifying, it returned. The presence of alkali is, therefore, inhibitory to the fuchsinophile reaction.

Another experiment with the cortex of a kidney confirmed this. Portions of the cortex were put into a 1 per cent. solution of NaOH for twenty hours. At the end of that time a slimy residue remained, from which (although with some difficulty) a series of films was prepared and stained. On microscopic examination they showed a non-fuchsinophile reticular stroma. The rest of the residue from the alkaline digest was now put into 10 per cent. HCl for eighteen hours, when films were again prepared from the residue. Microscopic examination now showed a fuchsinophile reticulum.

The Behaviour of Egg-White.—Though films made from untreated egg-white are not fuchsinophile, yet on treatment with HCl the reaction is given powerfully. Other acids such as acetic and lactic produce the same result ; in the case of lactic acid, however, the film is very difficult to manipulate on account of the sticky character of the acid in question.

The phenomenon thus appears to depend entirely upon the hydrion, the anion of the acid being of no importance. This is, of course, subject to the acid being sufficiently dissociable to give an adequate hydrion concentration. Allusion will be made to this matter again when the chemistry of formol-Müller fixation is discussed.

The Behaviour of certain Metallic Albuminates.—When a solution of egg-white is added to one of basic lead acetate or of mercuric chloride, a precipitate of metallic albuminate is produced in each case. The lead acetate solution is definitely alkaline to litmus : solutions of mercuric chloride in pure water are, on the contrary, always slightly acid, this being due to the liberation of free hydrochloric acid by hydrolysis. Precipitates formed in these solutions will necessarily contain small amounts of free alkali and free acid respectively. Films of each of these precipitates were prepared upon the same slide, stained and differentiated. The mercury albuminate showed a fuchsinophile reaction, while the lead albuminate did not.

The Chemistry of Formol-Muller Fixation.

The formol-Müller fluid used in these researches consists of Müller's fluid to which two per cent. of commercial formalin has been added. In considering the chemical action of cell fixation by its means we have to consider the action of—

1. Formaldehyde,
2. Potassium bichromate,
3. Sodium sulphate.

1. Formaldehyde.—Although commercial formalin only contains forty per cent. of formaldehyde, and of this a certain proportion always undergoes oxidation to formic acid, yet the fixing power of Müller's fluid (as regards granules) is enormously increased by the addition of so small a quantity as 2 per cent.

If a film of egg-white be prepared, and a portion treated with formalin, the treated part will be found to have acquired definitely fuchsinophile properties when compared with the rest of the film. Now the action of formaldehyde upon a neutral or even faintly alkaline protein is to substitute for the neutral or faintly alkaline reaction one of a distinctly acid character, the alteration in question being due to a conversion of the basic NH_2 groups in the protein molecule into neutral NCH_2 groups by the formaldehyde. Since proteins may be regarded as formed by the condensation of a series of amino-acids, it is clear that by eliminating the basic radicles the carboxyl groups are given free play, and the result is an acid reaction. Apart from theoretical considerations this fact may be demonstrated as follows: To a few c.c. of commercial formalin (which is always acid from the presence of formic acid) add a drop or two of an alcoholic solution of phenolphthalein (as indicator), then add just sufficient caustic soda solution to give a faint pink colour. Next, to a solution of Witte peptone add a little phenolphthalein and render just alkaline as before. On adding the alkaline formalin to the alkaline peptone solution the pink colour is gradually discharged, and a considerable amount of soda must be added to bring it back again. This method of neutralising the NH_2 groups of amino-acids is of course familiar to chemists as a useful method for direct titration.

2. Potassium Bichromate.—It is known that the chromates and bichromates are readily convertible. Thus addition of acid to a chromate produces a bichromate as in the following equation :—

$$2\ K_2CrO_4 + H_2SO_4 = K_2SO_4 + K_2Cr_2O_7 + H_2O.$$

Even weak acids such as acetic produce a similar effect. Conversely, on adding alkali to a solution of a bichromate, the orange colour of the latter is replaced by the yellow of the former :—

$$K_2Cr_2O_7 + 2\ KOH = 2\ K_2CrO_4 + H_2O.$$

A solution of the bichromate of a strong base is always acid, as owing to hydrolysis a partial formation of $H_2Cr_2O_7$ occurs, which dissociates into $H_2^{\cdot\cdot}$ and Cr_2O_7''. Any attempt at neutralising a solution of such a bichromate only results in the formation of a certain amount of chromate, according to the equation given above. Although the explanation is different it is noteworthy that a similar conversion of bichromate into chromate takes place in the fixation of tissues by Müller's fluid, and it is owing to the uselessness of chromates for fixation that the method is adopted of using large quantities of Müller and changing them frequently.

These relations between the bichromates and the chromates led to an extremely interesting and important result as far as the granules of the kidney cortex are concerned. A certain degree of fixation of these granules is possible by potassium bichromate alone. But it was also found that chromates, notoriously useless for fixing purposes, give granule results in no way inferior to those obtained by means of bichromates. This suggests that in the granule a conversion of chromate into bichromate has occurred, and since this change can take place only in the presence of free acid, strongly supports the view that the granule substance is of "acid protein" character.

From what has been said previously it will be seen that formalin would be a powerful adjuvant in producing the above result. That this is not due to admixture of formic acid is shown by the fact that formic-acid Müller does not fix the lymphocyte granules (the most difficult to fix), whereas formol-Müller does.

The relations existing between protein solutions, formalin, chromates and bichromates, may be demonstrated very simply *in vitro*. If a 20 per cent. solution of egg-white be added to a solution of potassium chromate, the solution remains yellow. On now adding *neutral* formalin, the orange colour of the bichromate appears, owing to the acidifying action of the formalin upon the protein. If allowed to stand the yellow colour returns, owing to the protein having combined with the CrO_3 of the $K_2Cr_2O_7$ and thereby leaving K_2CrO_4.

3. Sodium Sulphate.—This substance is commonly stated to assist the penetration of the bichromate, but as regards granule fixation we think it has another and more important rôle. We have found, as described in another paper in the present volume, that soaking the tissue in water for twenty-four hours previous to formol-Müller fixation has a markedly deleterious effect upon the fuchsinophile reaction of the granules. The phenomenon is most marked in the case of the lymphocyte granules, but is also noticeable in those of the kidney cortex. This fact strongly supports the view that the granule consists of protein in combination with an acid. Assuming that the lymphocyte granule, for example, consists of protein in combination with a comparatively feeble acid, the addition of water will liberate the acid in question, and the protein of the granule will combine with the hydroxidion of the water, thereby acquiring definitely basic instead of acidic characters, and losing its former fuchsinophile quality.

If, however, for water, sodium chloride solution be substituted the conditions are correspondingly modified. There is then a tendency towards double decomposition between the sodium chloride and the acid portion of the acid protein granule. Thus if B represent the protein and R the anion of the acid with which it is in combination, the process reduced to its simplest form may be represented as follows: $B.HR + NaCl \rightleftarrows B.HCl + NaR$. Owing to the more highly ionized character of the hydrochloric acid, the acidic character of B.HCl will be much more marked than that of B.HR, and consequently the fuchsinophile reaction will actually tend to be increased. In practice, however, this tendency to increase will be counteracted by the weakly basic character and high molecular weight of the protein molecule, since compounds of even strong acids with feeble bases tend to undergo hydrolysis when in dilute solution.

Sodium sulphate, as an easily dissociable salt of a strong acid, would produce an effect similar to that of sodium chloride. The sodium sulphate in formol-Müller therefore appears to us to reinforce the acidifying action we have already shown to be exerted by the bichromate and the formalin.

This action of sodium chloride or sodium sulphate can be readily demonstrated *in vitro*. To a solution of lactic acid add a trace of methyl orange; the solution becomes red. Then add sufficient egg-white solution to just bring back the original colour of the methyl orange. On now adding a solution of sodium chloride, the bright red colour characteristic of the presence of free acid reappears.

Methods of Differentiation for Altmann's Granules.—The differentiation of granules from the surrounding tissue when stained by acid fuchsin can be effected either by picric acid alcohol or by dilute alkalis. The process is essentially different in the two cases.

In the case of the picric acid solution, the stain is displaced from its tissue combination and unites with the picric acid. But affinity of the stain for the granules is more marked than for the rest of the cell constituents, and they consequently resist this displacement longer. Now picric acid solutions are definitely acid in character, and strongly fuchsinophile. The tenacity with which the granules hold the stain is therefore an argument that they, like picric acid, possess free hydrions. Combinations of alkali with acid fuchsin, on the other hand, are colourless, and differentiation by alkali depends upon the ready formation of this colourless compound in the relatively weakly acid intergranular substance, and its failure to form in the relatively highly acid granule.

To sum up, the evidence derived from the preceding experiments tends to show that the affinity of the various constituents of cytoplasm for aniline-acid-fuchsin is more or less proportional to their acidity, and consequently we conclude that the fuchsinophile granules of the kidney are—

1. Protein in character.
2. Possess a higher hydrion concentration than the rest of the cell substance in which they occur.

In other words, the Altmann's granules of the kidney are of an acid protein nature.

A NOTE ON THE EFFECTS OF VARIOUS REAGENTS ON THE DEMONSTRABILITY OF ALTMANN'S GRANULES.

By H. BECKTON and H. A. COLWELL.

It has been indicated elsewhere that while Altmann's granules can be demonstrated in lymphocytes, plasma-cells, fibroblasts, etc., after fixation in formol-Müller (formalin 1, Müller's fluid 49), but not after fixation with dilute formalin alone, those of liver cells, pancreas cells, kidney (cortex) cells, etc., can be shown after fixation in either fluid. In this paper are described the results found on examining the effects of various reagents on these granules. In some cases the tissue has been treated with formol-Müller after the reagent employed; in others the reagent has itself been considered as a possible fixing agent for the granules.

In the following the expression "disappearance of granules" must be taken as meaning simply that the granules seen on fixing in formol-Müller, etc., are not seen under the existing circumstances described. They may of course be present, but in modified form.

I.—Heat.

This is considered from two points of view: (*a*) as a method of fixing films for granule-staining, (*b*) as affecting tissues in bulk.

(*a*) On making film-preparations from various tissues and heating for a few minutes to 60° C., or even simply allowing them to dry, it is found on staining for Altmann's granules that the cells of liver, kidney (cortex), pancreas, and submaxillary gland show large, discrete, usually round or oval granules, while the cells of the connective-tissue group (lymphocytes, fibroblasts, etc.) do not show granules.

(*b*) A small piece of freshly-excised sheep's spleen was placed in a capsule in an incubator at 37° C. and small portions of this removed at intervals and fixed in formol-Müller. Those removed at the end of one hour and two hours showed lymphocyte granules as well, or very nearly as well, as a control piece placed directly in formol-Müller ; a piece removed at the end of three hours showed some diminution, and a piece removed at the end of four hours a very great diminution, in number of lymphocyte granules. These results were confirmed in the case of a piece of mouse's spleen ; the lymphocyte granules were very well seen after two hours' exposure to a temperature of 37° C., while after four hours' exposure they had almost all disappeared ; after six hours' exposure the disappearance was still more marked, and no lymphocyte granules at all were found in a piece removed at the end of 24 hours. It was noted that the granules of fibroblasts and endothelial cells were somewhat more persistent than those of lymphocytes.

This disappearance of Altmann's granules in the case of spleen is found to be still more rapid when the tissue is kept at a temperature of 55° C. In marked contrast is the fact that a piece of spleen kept at room-temperature for a day or even longer shows little or no loss of granules.

II.—X-Rays, etc.

It was found that small pieces of tissue exposed for an hour or two to X-rays showed no change as regards Altmann's granules. The effects of radium emanation are described in another paper in the present volume.

III.—Distilled Water. Aqueous Solutions of Sodium Chloride and Potassium Chloride.

An exposure for twenty-four hours to the action of distilled water causes the complete, or nearly complete, disappearance of Altmann's granules from all the cell species investigated. Watery solutions of sodium and potassium chlorides, however, act differently towards the different tissues. Thus lymphocytes showed Altmann's granules in a piece of sheep's spleen kept in 0·8 per cent. and 1·0 per cent. solutions of sodium chloride for twenty-four hours as well as in a freshly excised portion ; with 0·6 per cent. and 2·0 per cent. solutions the granule appearances were slightly, and with 0·5 per cent.

solutions markedly, inferior. Similar results were obtained with mouse's spleen. From pancreas 0·8 per cent. solution of sodium chloride causes complete, and from liver and kidney almost complete, disappearance of granules in twenty-four hours. In the case of potassium chloride the lymphocyte granules of spleen showed very well after twenty-four hours' stay in 0·5 per cent., 1·0 per cent., and 2·0 per cent. solutions, but not quite so well with 3·0 per cent., 4·0 per cent., and 5·0 per cent. solutions; with higher concentrations the granule appearances became poorer and general histological detail inferior.

IV.—Acids and Alkalis.

The actions of these are dealt with in another paper in the present volume. It is sufficient to state here that while alkalis of all strengths cause the disappearance of Altmann's granules from the various tissues investigated, and very dilute acids do the same, acid of medium concentration has different effects on different tissues. Thus 50 per cent. HCl, after prolonged action (e.g., several days) allows of demonstration of Altmann's granules in kidney (cortex cells), but not in spleen. Strong acid causes complete disintegration of tissues.

V.—Alcohol, Acetone, Ether, Chloroform.

After exposure for twenty-four hours to the action of any of these, either pure or mixed with water, Altmann's granules can no longer be demonstrated in cells of the connective-tissue group, either with or without subsequent treatment with formol-Müller. A very inferior fixation for granules can, however, be obtained for some other cell-species; thus granules may be seen in kidney cortex after acetone, or in liver after chloroform.

It is evident from the foregoing that operation-material placed in alcohol is thereby rendered useless as regards examination for Altmann's granules.

VI.—Formalin.

Here again a practical question arises: Can reliance be placed on the result of examination for Altmann's granules in the case of operation-material which has been preserved in formalin? It has been suggested in a previous paper in these Archives that the formol-Müller method depends upon fixation by formalin and subsequent mordanting by Müller's fluid. In order to find an answer to this question pieces of mouse's liver, spleen, and kidney were placed in formalin (5 per cent. made up with "physiological saline solution"—NaCl ·75 per cent.) for a day and subsequently treated with formol-Müller; tissues which had been kept in formalin for a year were also placed in formol-Müller. The former showed Altmann's granules brilliantly, as in material fixed directly in formol-Müller, in all the various cell-species involved; the latter not at all. Two further series (liver, spleen, kidney, duodenum, pancreas, submaxillary salivary and lymphatic glands) were kept for approximately a week and a month respectively in formalin (10 per cent., made up with NaCl. ·75 per cent.) and subsequently taken through formol-Müller. Lymphocyte granules after a week of formalin still appeared throughout the lymphoid tissue examined, but they were not so well marked as after direct fixation in formol-Müller or after one day in formalin, followed by formol-Müller; after a month in formalin they did not appear at all. Other granules showed appearances of more or less deterioration with time in formalin; of all the numerous species observed only the very large granules of the submaxillary gland appeared still very large, deeply stained and sharply defined at the end of a month.

It thus appears that operation-material placed in moderately thin slices in formalin is available for examination for Altmann's granules, provided that it be transferred to formol-Müller within a day or two.

OBSERVATIONS ON THE BLOOD AND BONE MARROW OF HEALTHY AND OF CARCINOMATOUS MICE.

By C. PRICE-JONES.

THIS research was undertaken to determine whether the blood of experimental carcinomatous mice presents any cytological variations from the blood of healthy mice, and whether, if variations exist, they can be traced to any altered conditions in the cellular constitution of the bone marrow. The subject appeared also to be of interest in connection with the now well-known fact that the blood of malignant disease in the human subject shows an increased number of large mononuclear lymphoid cells, since the blood of the normal mouse is especially characterised by a large number of these cells.

Examinations of blood and bone marrow were made of thirty-four mice, eighteen of which were healthy and sixteen cancerous. The animals were weighed, and, in the case of the mice with tumours, the tumour was dissected out and separately weighed, the real weights of the mice being estimated after deduction of the weight of the tumour.

The animals were anæsthetised by a mixture of chloroform and alcohol in equal parts (it was found that the mouse is very intolerant of pure chloroform, and dies too rapidly to permit a complete blood examination). The thorax was quickly opened and samples of blood were taken from the heart.

Hæmoglobin percentage estimations were made with a Gowers-Haldane standardised hæmoglobinometer; enumerations of red and white blood cells were made with Bürker's modification of the Thoma-Zeiss hæmocytometer, the diluting fluid being 10 per cent. formalin in ·8 per cent. salt solution, to which a few drops of saturated solution of methyl-violet were added, whereby the white cells are stained and may be counted in the same field as the red cells. The appearances of the red cells and the differential counts of the white cells were observed on films stained by Jenner's stain. The bone marrow was examined by the glycerine film method and stained with Jenner's stain. The spleens of twenty-six animals were weighed, and from each a glycerine film was prepared, and stained like the marrow films.

The Blood of Healthy Mice.

Unfortunately, it is not practicable to make day by day examination of the mouse's blood, since it is probably impossible, except, perhaps, by cardiac puncture, to obtain sufficient blood for a complete examination without killing the animal; so that to obtain a standard estimate of the cytology of the blood it is necessary to take an average of the results obtained from the examination of a number of different animals; and since in different individuals there are very great variations both in number and in type, the estimated normal standard cannot approach any high degree of accuracy. The one constant feature of mouse blood is the presence of varying amounts of polychromasia indicating varying degrees of red-cell regeneration and activity of the blood-cell forming organs; probably this is the chief cause of the cytological variations found in the blood. The degree and type of this variability did not appear to have any constant relation to the size of the mouse.

For the purpose of arriving at a fair estimate of the healthy mouse blood I have taken the average of the results obtained from thirteen of the eighteen animals examined, five mice being excluded on account of extreme variations; two were newly born, one showed an unusually high leucocytosis, one had eosinophilia, and one showed an extreme lymphoerythroblastic condition of its bone marrow.

The weight of these thirteen healthy mice averaged 17·9 grms. (Table I.). The hæmoglobin percentages of these mice varied from 48 to 114, and averaged 77·84. Table I. shows that the size of the animal had apparently no connection with this variation. The mouse with 114 per cent. weighed 23 grms., but the largest animal, 34 grms., had only 82 per cent.; a mouse weighing only 13 grms. had 104 per cent.; and the mouse with the lowest percentage, 48, weighed

17 grms. Goodall * gives the hæmoglobin percentage for the white mouse as 90.

The red cells varied in number from 5·7 to 10·8, and averaged 8·73 million per c.mm.; the colour index averaged 0·48. Goodall finds 10·85 million red cells per c.mm., so that his colour index is 0·43.

The diameter of the red cells was not measured, but Goodall's estimate is 5·6 μ.

As stated above, there was in all the bloods a certain amount of polychromasia of the red cells, which in some cases was estimated at about 20 per cent., in others only 5 per cent. Basophilia of the red cells, so commonly observed in the blood of rabbits and guinea-pigs in anæmic conditions, was never seen in any film.

With two exceptions, nucleated red cells were found in the blood films of all these healthy mice: a further indication of the activity of the blood-forming organs, and of the demand of the blood for fresh cells to replace those lost by destruction. In the thirteen mice, in counts of 300 white cells each, twenty-eight nucleated red cells were enumerated.

The white cells varied in number from 3,200 to 16,400, and averaged 8,220 per c.mm. Goodall finds 5,000 per c.mm.

These cells include lymphocytes, large mononuclear lymphoids, polymorphonuclear leucocytes, eosinophil leucocytes, and mast-cells.

The lymphocytes are the most numerous variety of white cell. The average relative number was 59·76 per cent., or an average absolute number of 4,911 per c.mm. These cells measure about 7 μ in diameter, and resemble in morphology and in staining the lymphocytes of man and other animals.

The large mononuclear lymphoid cells are very various in appearance; they exhibit two chief morphological types: (*a*) Those cells with a round or oval, undivided, sometimes eccentrically placed nucleus which usually stains deeply basophil, though less so than the nucleus of the lymphocyte; the cytoplasm is absolutely and relatively greater than that of the lymphocyte, and is either unstained or appears faintly basophil, and contains various sized, deeply basophil, granular-looking bodies, which are generally regarded as nodes of cytoreticulum. (*b*) Those cells which formerly were considered as transitional to polymorphonuclear leucocytes; these cells are usually larger than the preceding: the nucleus is single, deeply notched or indented and sometimes divided, and is stained faintly basophil. Both these types of cell have large diameters, often as much as 20 μ, but the size is variable and in some cases only about 10 μ.

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* "Journ. Path. and Bact.," vol. xiv., p. 195.

Intermediate forms between these two forms are always present, and all the varieties have been classed together into one group.

The average relative number of these cells was 21·55 per cent., or an average absolute number of 1,771 per c.mm. This high figure contrasts with those of other animals (man 4–8 per cent.; monkey, 5 per cent.; rabbit, 6–10 per cent.; rat, 4–10 per cent.); it is probably connected with the active blood destruction and regeneration that occurs in the mouse. These cells are largely concerned in phagocytosis, and in the films prepared from the spleens and marrows of these mice phagocytes containing red cells were very commonly met with. High percentage counts of these cells are recorded in the blood of protozoal disease, in pernicious anæmia, in tubercle, and in the blood of persons suffering from malignant disease.

The polymorphonuclear leucocytes are relatively few in number, averaging 16·96 per cent., and having an average absolute number of 1,394 per c.mm. These cells are about 10 μ in diameter; they have a deeply basophil stained, often much twisted and divided nucleus; the cytoplasm usually appears unstained, but may be very faintly basophil; as a rule no granules were visible by the staining method employed, but occasionally a few very fine neutrophil granules could be seen. It is interesting to note that in the glycerine film of the marrow, the cytoplasm of these cells is stained faintly oxyphil, and granules are more often recognised than in the blood films; I have also noted this effect in marrow films from the dormouse, and also from the cat, in both of which animals the granules of these cells are rarely to be distinguished in blood films.

The eosinophil leucocytes averaged 1·48 per cent. of all the white cells, having an average absolute number of 122 per c.mm. They are usually about the same size or rather smaller than the preceding; the nucleus stains deeply basophil and is much divided, or may be ring-shaped; the cytoplasm is faintly basophil, and contains a variable number of coarse coccal-shaped granules which stain well with eosin.

Mast cells were only rarely met with, and were only enumerated in counts of 300 white cells in five of the thirteen mice. They are large cells, about 15 μ in diameter; the nucleus, which stains faintly basophil, is undivided, and surrounded, as it were, by a broad halo of varying sized, coarse, deeply stained metachromatic basophil granules.

The Bone Marrow.

The cells of the bone marrow belong to three main groups:—1. Lymphoid cells which give rise to red cells, lymphocytes and leucocytes; 2. Leucoid cells, which are derived from the preceding, and which are granular myelocytes destined to form leucocytes; 3. Nucleated red cells which are also formed from 1, and which comprise various transitional forms, stages in the development of red corpuscles.

The accompanying phylogenetic diagram (pp. 62, 63) shows the relations of these cells, the detailed description of which I have given in "The Journal of Pathology and Bacteriology," vol. xv, 1910, p. 4.

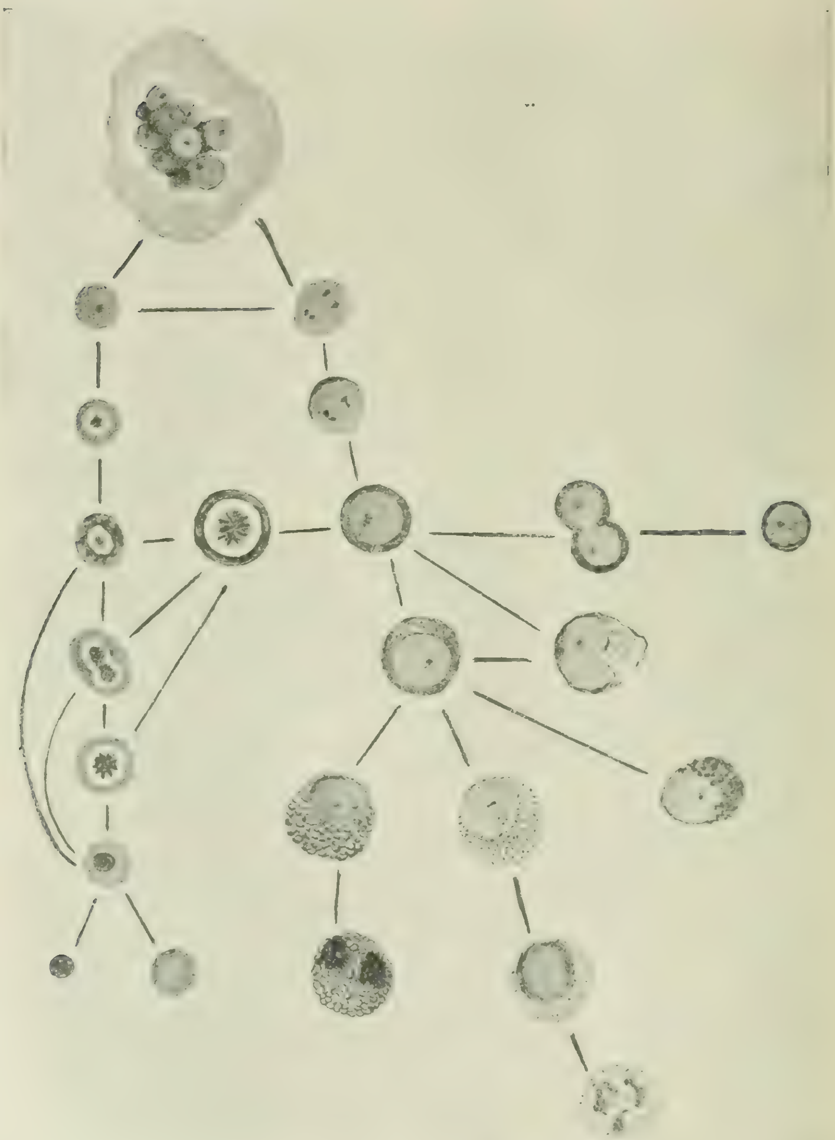
The marrow cells of the mouse conform generally to the above scheme, but the development of the polymorphonuclear leucocytes presents a variation from the common type. According to the phylogenetic plan which applies to the marrows of most animals I have examined, the polymorphonuclear leucocytes arise directly from fine oxyphil (neutrophil) granular myelocytes; the nucleus of this cell (granular myelocyte) acquires a less stained central area, suggesting a thinning or degeneration which is associated with a disappearance of the nucleus and a deeper staining of the nuclear periphery, a condition that leads to the appearance of a granular cell with a ring-shaped nucleus, which, becoming twisted and broken, eventually constitutes the polymorphous nucleus of the leucocyte. The granular myelocyte has arisen from the large primitive lymphocyte (myeloblast) by granule formation in its peripheral margin (loc. cit.). In the mouse the leucocyte seems to be developed directly from the primitive lymphocyte (myeloblast) which, before the granulation of the margin has proceeded to any great degree, shows a thinning and often, as it were, a central perforation of its nucleus. Numerous transitional forms are seen between this condition of cell and one in which the margin has ceased to stain basophil and has become indistinctly granular, often of a faintly oxyphil tint, and enclosing an irregular ring-shaped nucleus; this ring-shaped nucleus becomes more compact and fragmented and passes out into the blood as a polymorphonuclear leucocyte.

The coarse eosinophil leucocyte appears to develop from a granular myelocyte, a cell with a single nucleus surrounded by coarse eosin-stained granules, and transitional forms to this from the large primitive lymphocyte (myeloblast) are commonly met with; but I have seen occasionally a large primitive lymphocyte with a "perforated" nucleus and coarse oxyphil granules forming in the margin, suggesting that the eosinophil leucocyte may also be formed directly from the large primitive lymphocyte.

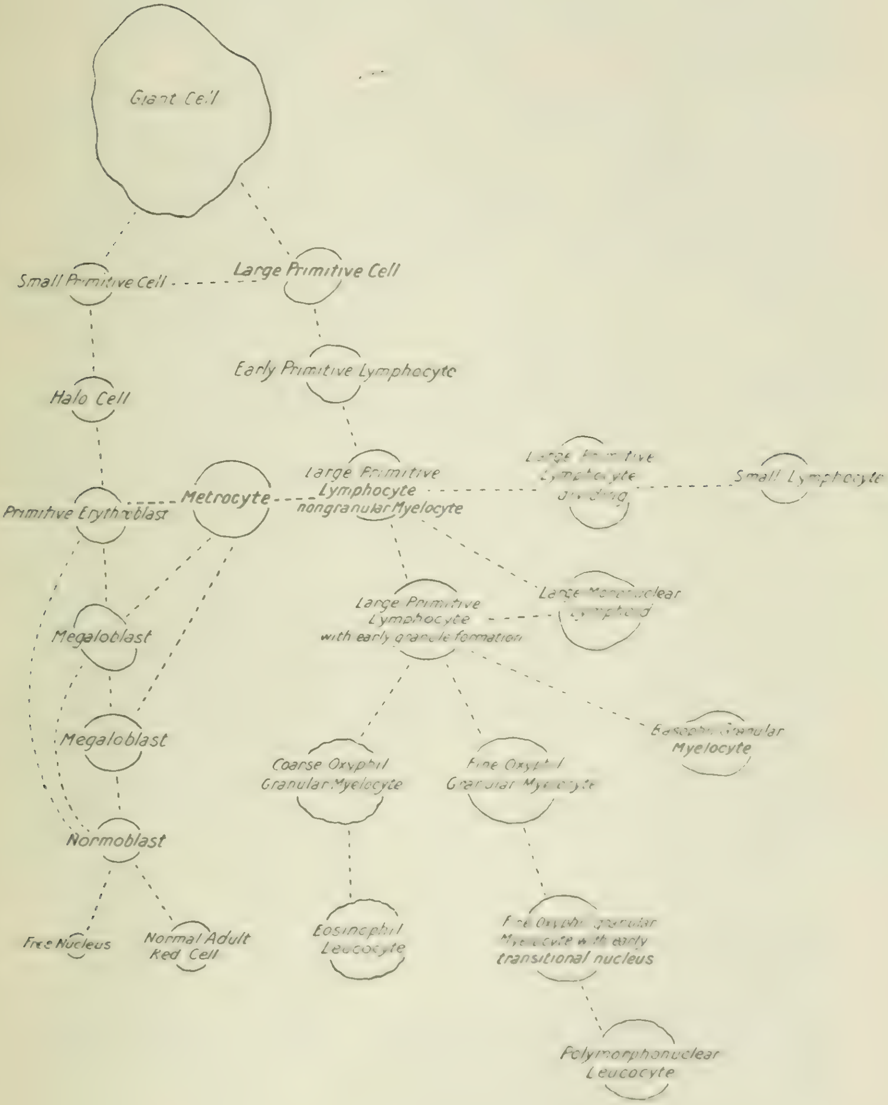
The cellular percentages obtained from the marrow films of thirteen healthy mice showed very great and irregular variations. It seems probable that these variations can be explained by the fact that the spleens of these animals were histologically myeloid in character, indicating that this organ was actively engaged in blood cell formation. This myeloid condition of the spleen seemed to vary in degree with different mice, and is probably complementary or supplementary to the activity of the marrow. I have made no numerical analyses of the cells from the spleen films.

In classifying the cells of the marrow I have adopted the practice of grouping all doubtful transitional cells with that class which is regarded as more primitive according to the scheme of development; the result of this procedure is that, owing to the absence of true finely oxyphil granular myelocytes, the percentages of the large primitive lymphocytes are increased, as explained above; the total percentages of the lymphoid cells is therefore relatively much greater than is commonly met with in other animals.

The nucleated red cells were counted simultaneously with the white cells and their percentage relation to these cells determined.



The Phylogeny of cells in Bone Marrow (diagrammatic). (From the "Journ. of Path. and Bact.," vol. xv.)


Giant Cell
Small Primitive Cell
Large Primitive Cell
Early Primitive Lymphocyte
Halo Cell
Large Primitive Lymphocyte dividing
Small Lymphocyte
Primitive Erythroblast
Metrocyte
Large Primitive Lymphocyte nongranular Myelocyte
Large Mononuclear Lymphoid
Megaloblast
Large Primitive Lymphocyte with early granule formation
Megaloblast
Basophil Granular Myelocyte
Coarse Oxyphil Granular Myelocyte
Fine Oxyphil Granular Myelocyte
Normoblast
Free Nucleus
Normal Adult Red Cell
Eosinophil Leucocyte
Fine Oxyphil granular Myelocyte with early transitional nucleus
Polymorphonuclear Leucocyte

Without going into details which may be studied in Table V., it appears that in healthy mice the average percentage of lymphoid cells is 58·32, of leucoid cells 41·44, and the number of nucleated red cells is about 27 to every 100 of the white cells counted.

This somewhat high percentage of lymphoid cells, as explained above, is due in part to the absence of fine granular myelocytes, but it is also due to the high percentage of large mononuclear lymphoid cells, 6·86, which in most marrows does not exceed 1 per cent., and which is interesting in connection with the large number of these cells in the blood.

The percentage of leucoid cells is correspondingly low, but includes a large percentage of polymorphonuclear leucocytes, 35 per cent., which contrasts with the percentages of these cells found in the marrow of other animals, man 5 per cent., rabbit 5 per cent., dormouse 15 per cent., rat 13–20 per cent.; and it is especially striking when considered with the fact that the average percentage of these cells in the blood of the 13 mice was only 16·96.

The nucleated red cells, as in other marrows, show a preponderance of free nuclei—13·89 or 51·4 per cent. of all the nucleated red cells. In all cases, with one exception, metrocytes were met with, which is unusual in healthy marrows; these cells, together with their daughter cells the megaloblasts, number 3·94 or 14·6 per cent. The remaining nucleated red cells are the normoblasts and their less mature forms the primitive erythroblasts, and these together constitute 33·23 per cent. of the nucleated red cell.

Speaking generally, the average of these marrows shows a total larger number of nucleated red cells than is commonly met with in the marrows of other animals, and this increase is especially due to an increase in the numbers of the free nuclei, or, in other words, the output of mature red cells from the marrow of the mouse is greater than occurs usually with other animals under healthy conditions.

The Spleen.

The spleens varied in weight from 0·04 to 0·24 grms.; the total weight of the spleens of the 11 healthy mice amounted to

1·42 grms. ; since the total weight of the mice was 199 grms. 100 grms. weight of mouse has 0·71 grms. weight of spleen.

Glycerine films prepared from these spleens showed besides large numbers of small lymphocytes, varying numbers of myeloid cells, and closely resembled marrow films. No differential counts of these cells were made.

The Blood of Carcinomatous Mice.

Blood examinations were made of 16 mice which had carcinomatous tumours. Of these 3 were rejected on account of errors in staining technique. The weight of the 13 mice after deducting the weights of the tumours was 257 grms., or an average of 19·76 grms.

As was the case in the bloods of the healthy mice, great variations occurred and variable amounts of polychromasia were always observed. The details of these blood examinations are given in Tables II and IV, but for the sake of brevity and the more ready appreciation of their value, the total results are set out in the following columns, where they are contrasted with the results obtained from the similar examinations of the bloods of the 13 healthy mice.

Averages.	Healthy Mice.	Cancer Mice.	—
Hb.	77·84	73·46	Decrease of 4·38 %
Colour index ...	0·48	0·48	—
Red cells, per c.mm.	8·73 millions	7·56 millions	Decrease of 1·17 m.
White cells ,,	8·22 thousands	14·69 thousands	Increase of 6·47 th.
Lymphocytes ,,	4,911	6,325	,, 1,414
Mononuclears ,,	1,771	4,733	,, 2,962
Polymorphs ,,	1,394	3,394	,, 2,000
Eosinophils ,,	122	210	,, 88
Mast cells ,,	9	2	Decrease of 7
Nucleated red cells per 300 white cells	2·1	0·46	,, 1·64

From this it appears that in cancer mice there is a slight diminution in the number of red cells per c.mm., and in the hæmoglobin percentage, and a very marked increase in the number of white cells, amounting to about 70 per cent. This increase in white cells is especially accounted for by the increase of large mononuclear lymphoid cells, about 160 per cent., and of polymorphonuclear leucocytes, about 140 per cent. Finally, whereas with the healthy mice in counts of

300 white cells each there was a total of 28 (average 2·1) nucleated red cells, 3 mice only showing none of these cells, with the cancer mice in similar counts there was a total of 6 (average 0·46) nucleated red cells, and in the films of 9 mice none of these cells were observed.

In Tables III and IV details are given of the absolute numbers of white cells per c.mm.; inasmuch as the percentages in individual cases are not complete percentages, but mostly sum to only 99 and a fraction, there are slight discrepancies between the figures derived from the average of the percentages and those derived from the average of the absolute numbers. If, however, the weights of the two sets of mice are considered, and if each set is reduced to a common denominator of the number of white cells per c.mm. in 100 grms. of mouse, the figures set out in the following columns show results that closely agree with those arrived at above.

Total Numbers of	Healthy Mice.	Cancer Mice.	Per cent.
White cells per c.mm. ...	45,950	78,108	Increase over 60
Lymphocytes ,, ...	28,389	31,578	,, ,, 10
Mononuclears ,, ...	9,386	24,441	,, ,, 160
Polymorphs ,, ...	7,316	17,163	,, ,, 130
Eosinophils ,, ...	852	1,017	,, ,, 10
Mast cells ,, ...	115	15	Decrease

THE BONE MARROW OF CANCER MICE.

The marrow films of the 13 cancer mice, like those of the healthy mice, showed great variations in the relative numbers of the different cells, and the cause is presumably the same, namely the variable myeloid condition of the spleen.

The results of the examination of these marrows are given in Table VI. From this it does not seem that the average percentages of the cells differ very greatly from those found in the healthy mice. There is a slight increase in the total percentage of lymphoid cells, due chiefly to increases in the large primitive lymphocytes, small lymphocytes, and large mononuclear cells, and there is a small increase of nucleated red cells accounted for chiefly by an increase of the free nuclei.

The increase of the large primitive lymphocytes, which include early forms of granular leucocytes and transitional stages to large mononuclear cells, and also the increase of mature large mononuclear cells might have been expected in view of the increased numbers of polymorphonuclear leucocytes and large mononuclear cells in the blood of these mice. The leucoid cells are correspondingly fewer, due apparently chiefly to the lower percentage of polymorphonuclear leucocytes, which at first sight seems to imply that fewer of these cells are being formed in the marrow, but this is not necessarily the case. The relatively lower percentage is explained partly by the relatively higher percentage of the lymphoid cells, and partly by the fact that since more polymorphonuclear leucocytes are in the circulation, there will be fewer retained in the marrow. The same argument applies to the large mononuclear cells, which, although they appear to be increased in the marrow by about 2 per cent., are probably really being formed in much more increased numbers, since there are many more required by the circulating blood and therefore the marrow is relatively depleted of these cells.

The slight increase in the number of nucleated red cells, due to the free nuclei, is interesting in connection with the fact that fewer nucleated red cells were found in the blood of these animals. It seems to suggest that the marrow is turning out fewer immature red cells into the circulation, and that therefore relatively more free nuclei remain in the marrow, or in other words the blood appears to be making a less urgent demand on the marrow. But from the percentage numbers of the normoblasts and megaloblasts it is seen that the erythroblastic factor of the marrow is rather more, or certainly not less active than in the healthy marrows, and further when the definite though slight anæmia indicated by the diminished number of red cells per c.mm. and the lowered hæmoglobin percentage, is considered, it would be reasonable to expect a more urgent demand by the blood, a greater erythroblastic activity, and a greater number of nucleated red cells in the circulation. It seems very probable, therefore, that the marrow is being helped by some other source of blood cell formation, and there is little doubt that this is provided by the spleen.

THE SPLEEN OF CANCER MICE.

The spleens of these mice were generally bigger, firmer in texture and darker in colour. They varied in weight from 0·12 to 0·74 grms.; the total weight of the spleens of 11 cancer mice was 3·50 grms.; since the total weight of these mice was 225 grms., 100 grms. weight of cancer mouse has 1·55 grms. of spleen, or, in other words, the spleen of the cancer mouse averages more than twice the weight of the spleen of the healthy mouse.†

It is noticeable that the two heaviest spleens occurred in the mice with the largest tumours.

Glycerine films prepared from these spleens showed, like those from the healthy mice, varying but always considerable amounts of myeloid cells. The assumption may, therefore, be made that there is about twice as much myeloid activity proceeding in the spleens of the cancer mice as there is in the spleens of the healthy mice, and that the marrow activity is correspondingly relieved.

SUMMARY.

The conclusions arrived at from this inquiry into the blood of carcinomatous mice may be summed up as follows:—

1. The blood shows a slight degree of anæmia, as indicated by a diminished number of red cells per c.mm., and a lowered hæmoglobin percentage.
2. There is a well-marked leucocytosis.
3. There is a relative and absolute great increase in the number of the large mononuclear lymphoid cells.
4. There is a relative and absolute great increase in the number of the polymorphonuclear leucocytes.
5. The spleens of healthy mice have a myeloid character.
6. The spleens of cancer mice have twice the average weight of the spleens of healthy mice.
7. That but little change can be observed in the marrows of cancer mice as compared with those of healthy mice, since owing to the increased myeloid activity of the spleens of cancer mice, any increased activity in the marrow is either masked or obviated.

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† It appears from the figures given by Medigreceanu ("Berliner klinische Wochenschrift," 1910, No. 13) that 100 grms. of normal mouse, less weight of intestinal contents has 1·03 grms. of spleen, and 100 grms. of cancerous mouse has 2·25 grms. of spleen, or more than twice the weight.

TABLE I.—BLOOD OF THIRTEEN HEALTHY MICE.

No.	Weight.	Hb. per cent.	Colour index.	Red cells in millions per c.mm.	White cells in thousands per c.mm.	Lymphocytes per cent.	Large mononuclear per cent.	Polymorph leucocytes per cent.	Eosinophil per cent.	Mast cells per cent.	Nucleated red cells per 300 w.c.	SPLEENS. Weight.
1	21	92	·46	10·1	5·5	62·0	11·0	24·0	2·6	0·3	9	—
4	13	106	·55	9·6	3·2	56·2	33·8	8·2	1·4	0·4	3	—
5	13	76	·43	8·8	10·6	73·0	13·0	11·6	2·3	0	1	0·160
6	13	104	·51	10·1	4·0	59·3	31·0	9·3	0·3	0	2	0·070
7	23	114	·52	10·8	9·7	68·0	10·6	15·0	6·0	0·3	0	0·070
8	16	98	·57	8·6	4·4	53·6	12·3	34·0	0	0	2	0·040
9	17	94	·51	9·2	10·4	57·3	30·0	11·3	1·0	0·3	0	0·095
10	34	82	·52	7·6	9·3	45·8	23·8	28·6	1·8	0	6	0·175
13	20	76	·50	7·4	16·4	78·3	12·6	8·3	0·6	0	1	0·240
15	15	76	·52	7·2	9·5	44·3	35·6	19·6	0·3	0	2	0·220
16	17	60	·32	9·3	8·6	69·6	10·6	17·6	2·0	0	1	0·080
17	14	86	·47	9·1	9·3	59·0	28·6	11·0	1·0	0·3	1	0·085
18	17	48	·42	5·7	6·0	50·6	27·3	22·0	0	0	0	0·190
Total ... 13	233	1,012	6·30	113·5	106·9	777·0	280·2	220·5	19·3	1·6	28	1·420
Average —	17·9	77·84	0·48	8·73	8·22	59·76	21·55	16·96	1·48	0·12	2·1	—
Absolute number of white cells per c.mm., calculated from the averages					8,220	4,911	1,771	1,394	122	9	—	Per 100 grms. weight of mouse. 0·713

TABLE II.—BLOOD OF THIRTEEN CARCINOMATOUS MICE.

No.	Weight, less tumour.	Weight of tumour.	Hb. per cent.	Colour index.	Red cells in millions per c.mm.	White cells in thousands per c.mm.	Lymphocytes per cent.	Large mononuclear per cent.	Polymorph leucocytes per cent.	Eosinophil per cent.	Mast cells per cent.	Nucleated red cells per 300 w.c.	SPLEENS. Weight.
2	18	0·2	92	·64	7·1	14·4	23·6	56·3	17·0	3·0	0	0	—
3	14	1·5	90	·65	6·8	13·6	41·0	41·3	17·0	0·6	0	0	—
6	17	2·78	45	·50	4·5	8·0	37·6	25·3	36·8	0·1	0	0	0·740
7	34	1·97	85	·47	9·0	25·4	52·0	30·0	17·0	1·0	0	0	0·330
8	21	0·91	52	·34	7·6	17·8	48·3	25·0	26·6	0	0	3	0·200
9	23	1·54	98	·56	8·7	21·7	25·6	41·3	32·6	0·3	0	0	0·320
10	21	0·5	80	·56	7·1	10·6	55·0	22·6	18·3	4·0	0	0	0·250
11	16	2·24	92	·50	9·2	8·9	46·3	18·3	32·6	2·6	0	0	0·320
12	23	6·04	92	·48	9·6	20·9	46·0	24·6	26·0	3·3	0	1	0·510
13	21	0·13	65	·42	7·6	13·1	61·6	23·0	14·6	0·3	0·3	0	0·220
14	15	0·57	56	·40	7·0	16·2	27·0	42·6	29·6	0·6	0	0	0·120
15	16	0·44	52	·31	8·3	10·4	54·6	24·6	19·0	1·6	0	1	0·220
16	18	0·22	56	·48	5·8	10·0	41·3	44·0	13·3	1·3	0	1	0·270
Total ... 13	257	—	955	6·31	98·3	191·0	559·9	418·9	300·4	18·7	0·3	6	3·50
Average —	19·76	—	73·46	0·48	7·56	14·69	43·06	32·22	23·11	1·43	0·02	0·46	—
Absolute number of white cells per c.mm., calculated from the averages			—	—	—	14,690	6,325	4,733	3,394	210	2	--	Per 100 grms. weight of mouse. 1·58 Ratio of cancer spleen to healthy spleen.
Ratio of cancer mice to healthy mice, calculated from the averages			10 10·5	10 10	10 11·5	17 10	12 10	26 10	24 10	16 10	$\frac{10}{45}\left(\frac{\cdot 22}{10}\right)$	—	$\frac{22}{10}$

TABLE III.—SHOWING CALCULATED ABSOLUTE NUMBERS OF DIFFERENT WHITE CELLS PER C.MM. IN THIRTEEN HEALTHY MICE.

	No.	Weight in grms.	Number of white cells in thousands per c.mm.	Number of lymphocytes per c.mm.	Number of large mononuclear cells per c.mm.	Number of polymorphonuclear leucocytes per c.mm.	Number of eosinophil cells per c.mm.	Number of mast cells per c.mm.
	1	21	5·5	3,410	605	1,320	143	16
	4	13	3·2	1,798	1,081	262	44	12
	5	13	10·6	7,738	1,378	1,229	243	0
	6	13	4·0	2,372	1,240	372	12	0
	7	23	9·7	6,596	1,028	1,455	582	29
	8	16	4·4	2,358	549	1,496	0	0
	9	17	10·4	5,959	3,120	1,175	104	31
	10	34	9·3	4,259	2,213	2,659	167	0
	13	20	16·4	12,841	2,066	1,360	98	0
	15	15	9·5	4,208	3,382	1,862	28	0
	16	17	8·6	5,985	911	1,514	172	0
	17	14	9·3	5,487	2,659	1,023	93	27
	18	17	6·0	3,036	1,638	1,320	0	0
Total ...	13	233	106·9 (107)	66.047	21,870	17,047	1,986	115
Numbers per c.mm. in 100 grms. of mouse			45,950	28,389	9.386	7,316	852	49

TABLE IV.—SHOWING CALCULATED ABSOLUTE NUMBERS OF DIFFERENT WHITE CELLS PER C.MM. IN THIRTEEN CARCINOMATOUS MICE.

	No.	Weight of mouse, less tumour.	Weight of tumour.	White cells in thousands per c.mm.	Lymphocytes per c.mm.	Large mononuclear cells per c.mm.	Polymorphonuclear leucocytes per c.mm.	Eosinophil cells per c.mm.	Mast cells per c.mm.
	2	18	0·2	14·4	3,398	8,107	2,448	432	0
	3	14	1·5	13·6	5,576	5,616	2,312	81	0
	6	17	2·78	8·0	3,008	2,024	2,944	8	0
	7	34	1·97	25·4	13,208	7,620	4,312	254	0
	8	21	0·91	17·8	8,597	4,450	4,734	0	0
	9	23	1·54	21·7	5,555	8,962	7,074	65	0
	10	21	0·5	10·6	5,830	2,395	1,939	424	0
	11	16	2·24	8·9	4,120	1,628	2,901	231	0
	12	23	6·04	20·9	9,614	5,141	5.434	689	0
	13	21	0·13	13·1	8,069	3,013	1,912	39	39
	14	15	0·57	16·2	4,374	6,901	4,795	97	0
	15	16	0·44	10·4	5,678	2,558	1,976	166	0
	16	18	0·22	10·0	4,130	4,400	1,330	130	0
Total ...	13	257	—	191·0 (190·738)	81,157	62,815	44,111	2,616	39
Numbers per c.mm. in 100 grms. of cancer mouse				78·108	31,578	24,441	17.163	1,017	15
Compared with 100 grms. of healthy mouse...				+32·158	+3,189	+15,055	+ 9,847	+165	−34
Ratio of numbers in cancer mice to numbers in healthy mice, calculated on the numbers in 100 grms. of mouse				$\frac{16}{10}$	$\frac{11}{10}$	$\frac{26}{10}$	$\frac{23}{10}$	$\frac{11}{10}$	$\frac{10}{23}$

TABLE V.—MARROWS OF THIRTEEN HEALTHY MICE.

	No.	Small and large primitive and halo cells.	Large primitive lymphocytes (myeloblasts).	Small lymphocytes.	Large mononuclear cells.	Giant cells.	Total lymphoid cells.	Oxyphil granular myelocytes.	Basophil granular myelocytes.	Polymorpho-nuclear leucocytes.	Total leucoid cells.	Normoblasts and primitive erythroblasts.		Megaloblasts.		Metrocytes.		Free nuclei.		Total nucleated red cells.	
												No.	p. c.	No.	p. c.	No.	p. c.	No.	p. c.	No.	p. c.
	1	23·0	24·2	1·6	2·8	0·8	52·4	4·0	0	43·6	47·6	59	11·8	23	4·6	2	0·4	96	19·2	180	36·0
	4	22·9	19·2	0·7	5·5	1·0	49·3	10·5	0·2	37·7	48·4	61	15·2	18	4·5	8	2·0	97	24·2	184	46·0
	5	18·4	30·2	1·0	4·8	0·6	55·0	12·2	0	32·8	45·0	47	9·4	13	2·6	14	2·8	28	5·6	102	20·4
	6	23·7	39·7	4·0	6·2	0·5	74·1	6·2	0·2	19·2	25·6	39	9·7	12	3·0	7	1·7	44	11·0	102	25·5
	7	20·2	16·2	2·6	4·4	0·8	44·2	7·0	0	48·8	55·8	38	7·6	12	2·4	3	0·6	36	7·2	89	17·8
	8	24·2	17·4	4·5	4·0	1·2	51·3	7·5	0	41·0	48·5	29	7·2	20	5·0	2	0·5	40	8·0	91	22·7
	9	26·2	17·0	5·0	7·0	0·7	55·9	5·7	0	38·2	43·9	27	6·7	11	2·7	3	0·7	74	18·5	115	28·7
	10	8·6	15·4	5·0	11·4	0·6	41·0	5·6	0	53·4	59·0	17	3·4	5	1·0	1	0·2	36	7·2	59	11·8
	13	21·2	23·4	9·0	12·2	0·8	66·6	10·4	0·2	22·8	33·4	36	7·2	6	1·2	0	0	88	17·6	130	26·0
	15	6·0	24·8	2·6	12·2	0·4	46·0	9·2	0	44·8	54·0	33	6·6	7	1·4	8	1·6	31	6·2	79	15·8
	16	16·8	35·4	14·6	6·0	0·6	73·4	1·2	0	25·4	26·6	55	11·0	12	2·4	15	3·0	92	18·4	174	34·8
	17	20·2	29·2	13·4	6·2	0·6	69·6	1·6	0	28·8	30·4	47	9·4	10	2·0	5	1·0	105	21·0	167	33·4
	18	15·6	48·2	8·2	6·6	0·8	79·4	2·8	0	17·8	20·6	58	11·6	13	2·6	7	1·4	84	16·5	162	32·4
Total ...	-	247·0	3,403	72·2	89·3	9·4	758·2	83·9	0·60	454·3	538·8	546	(116·8)	162	(35·4)	75	(15·9)	851	(180·6)	1,634	(351·3)
Average p. c. per mouse	—	19·0	26·17	5·55	6·86	0·72	58·32	6·45	0·04	34·94	41·44	—	8·98	—	2·72	—	1·22	—	13·89	—	27·02
P. c. of nucleated red cells	—	—	—	—	—	—	—	—	—	—	—	—	33·23	—	10·06	—	4·55	—	51·40	—	99·24

TABLE VI.—MARROWS OF THIRTEEN CANCER MICE.

	No.	Small and large primitive and halo cells.	Large primitive lymphocytes (myeloblasts).	Small lymphocytes.	Large mononuclear cells.	Giant cells.	Total lymphoid cells.	Oxyphil myelocytes.	Basophil myelocytes.	Polymorph leucocytes.	Total leucoid cells.	Normoblasts and primitive erythroblasts.		Megaloblasts.		Metrocytes.		Free nuclei.		Total nucleated red cells.	
												No.	p. c.	No.	p. c.	No.	p. c.	No.	p. c.	No.	p. c.
	2	21·8	21·8	3·6	1·8	1·5	50·5	3·6	0	45·8	49·4	52	10·4	36	7·2	4	0·8	97	19·4	189	37·8
	3	28·6	28·2	1·8	5·0	1·2	64·8	7·6	0·6	27·0	35·2	70	14·0	32	6·4	4	0·8	105	21·0	211	42·2
	6	23·9	21·3	8·0	12·6	1·0	66·8	3·0	0	30·0	33·0	31	10·3	4	1·3	0	0	42	14·0	77	25·6
	7	20·0	22·7	11·0	6·2	0·2	60·1	8·7	0	31·0	39·7	27	6·7	0	0	1	0·2	62	15·5	90	22·5
	8	19·0	15·8	4·8	13·2	1·4	54·2	7·0	0·6	38·2	45·8	38	7·6	8	1·6	3	0·6	99	19·8	148	29·6
	9	13·6	16·6	8·3	11·3	1·0	50·8	6·3	0	42·6	48·9	23	7·6	11	3·6	1	0·2	44	14·6	79	26·3
	10	22·6	33·0	8·4	7·0	0·6	71·6	9·8	0	18·6	28·4	65	13·0	20	4·0	11	2·2	72	14·4	168	33·6
	11	10·2	25·2	6·6	5·4	0·8	48·2	9·6	0	42·2	51·8	45	9·0	18	3·6	4	0·8	59	11·8	126	25·2
	12	25·6	24·6	6·8	14·4	1·2	72·6	4·6	0	22·8	27·4	52	10·4	19	3·8	16	3·2	122	24·4	209	41·8
	13	19·4	31·2	11·7	5·7	0·5	68·5	6·2	0	25·2	31·4	16	4·0	5	1·2	5	1·2	45	11·2	71	17·7
	14	10·8	37·6	8·0	11·8	0·4	68·6	2·2	0·2	29·0	31·4	84	16·8	26	5·2	4	0·8	80	16·0	194	38·8
	15	19·2	40·4	9·8	10·6	2·2	82·2	6·4	0	11·2	17·6	77	15·4	31	6·2	7	1·4	108	21·6	223	44·6
	16	13·2	50·0	7·4	7·2	0·8	78·6	3·8	0	17·6	21·4	31	6·2	13	2·6	6	1·2	45	9·0	95	19·0
Total	—	247·9	368·4	96·2	112·2	12·8	837·5	78·8	1·4	381·2	461·4	611	(131·4)	223	(46·7)	66	(13·4)	980	(212·7)	1,880	404·7
Average p. c. per mouse ...	—	19·06	28·33	7·40	8·63	0·98	64·42	6·06	0·10	29·32	35·49	—	10·10	—	3·59	-	1·03	—	16·36	—	31·13
P. c. of nucleated red cells	—	—	—	—	—	—	—	—	—	—	—	—	32·44	—	11·53	—	3·30	—	52·55	—	99·82

THE CYTOLOGY OF THE BLOOD IN MALIGNANT DISEASE OF MAN.

(Second Communication.)

By C. PRICE JONES.

With the preceding account of the blood in cancer mice, the following summary of the results obtained from the examination of the blood of thirty cases of human cancer form an interesting and instructive comparison. Details of these cases were published ten years ago in Vol. I of these Archives, but it seemed desirable, in view of the changes and advances in hæmatological knowledge that have taken place since then, to reconsider the results, and to endeavour, by presenting them in a different form, to arrive at more definite conclusions.

In the following tables the red cells are given in millions, the white cells in thousands, and the white cell varieties in absolute numbers per c.mm., respectively.

Carcinoma of the Breast.

No.	Red Cells.	White Cells.	Lymphocytes.	Mononuclear Cells.	Polymorphonuclear Leucocytes.
1	3·8	15·0	1,650	1,882	10,575
2	3·86	10·4	1,480	1,276	7,238
3	5·0	10·2	1,958	1,569	6,558
4	4·45	6·0	820	623	4,525
5	4·79	14·3	1,023	1,119	11,824
6	4·47	7·1	1,943	648	4,132
7	2·8	9·3	1,889	1,818	5,350
8	4·3	12·1	2,165	1,881	7,917
9	4·66	9·5	2,028	1,106	5,917
Total - -	38·13	93·9	14,956	11,922	64,036
Average -	4·23	10·4	1,661	1,324	7,115
Normal Average.	4·5	7·5	1,500	500	5,000
Difference -	−·27	+ 2·9	+ 161	+ 824	+2,115
Per cent. -	− 6	38	10	164	42

From this table it appears that the number of red cells in these cases is diminished on an average by 6 per cent.; the number of white cells per c.mm. is increased 38 per cent.; the lymphocytes are increased by 161 per c.mm., or 10 per cent. The large mononuclear cells are increased 164 per cent., and the polymorphonuclear leucocytes 42 per cent.

CARCINOMA OF THE STOMACH.

No.	Red Cells.	White Cells.	Lymphocytes.	Mononuclear Cells.	Polymorphonuclear Leucocytes.
1	1·46	24·5	4,900	6,487	12.436
2	3·40	8·7	1,530	965	6,190
3	2·7	13·9	1,444	865	10,716
4	4·32	9·2	1,764	881	6,403
5	3·68	7·3	2,020	797	4,314
6	3·62	24·5	1,499	1,878	20,798
7	4·78	9·6	1,190	670	7,747

Cases 1 and 6 were only examined on one occasion, and that was on the day before death, so that it seems fair for purpose of average to exclude them from the totals, especially as they show extreme figures compared with the other cases.

No.	Red Cells.	White Cells.	Lymphocytes.	Mononuclear Cells.	Polymorphonuclear Leucocytes.
Total - -	18·88	48·7	7,948	4,178	35,370
Average -	3·77	9·7	1,589	835	7,070
Normal Average.	4·5	7·5	1,500	500	5,000
Difference -	– 0·73	+ 2·7	+ 89	+ 335	+ 2,070
Per cent. -	– 16	37	6	67	41

From these figures it is seen that the diminution in number of the red cells is more (10 per cent.) than in the breast cases, and that the increase of white cells is the same, but the increase of large mononuclear cells is much less.

CARCINOMA OF THE INTESTINE.

No.	Red Cells.	White Cells.	Lymphocytes.	Mononuclear Cells.	Polymorpho-nuclear Leucocytes.
1	5·37	32·5	1,390	1,974	19,008
2	4·0	6·7	1,590	918	3,948
Total - -	9·37	29·2	2.980	2,892	22,956
Average -	4·6	14·6	1,490	1,446	11,478
Difference -	0	+ 7·1	− 10	+ 946	+ 6,478
Per cent. -	0	92	—	189	129

These two cases, of which only two examinations each were made, are so different in the numbers of their white cells per c.mm. that the averages obtained cannot be regarded as representative.

CARCINOMA OF THE GENERATIVE ORGANS.

No.	Red Cells.	White Cells.	Lymphocytes.	Mononuclear Cells.	Polymorpho-nuclear Leucocytes.
1	2·98	12·8	1,527	1,583	8,925
2	3·4	26·1	2,589	2,969	19,741
3	4·3	8·7	1,570	775	6,201
4	4·69	13·8	2,210	1,591	9,807
5	5·44	10·01	2,621	1,439	5,517
Total - -	20·81	71·41	10,517	8,357	50,191
Average -	4·16	14·28	2,103	1,671	10,038
Normal Average.	4·5	7·5	1,500	500	5,000
Difference -	− 0·34	+ 6·78	+ 1,603	+ 1,171	+ 5,038
Per cent. -	− 7	90	107	234	100

These cases show a much greater percentage increase in the number of white cells than those of the other series; this is probably connected with the various septic infections that commonly accompany these cases. It is also noticeable that although the increase of the polymorphonuclear leucocytes is large, the increase in the mononuclear cells is relatively very much greater.

Carcinoma of the Tongue.

No.	Red Cells.	White Cells.	Lymphocytes.	Mononuclear Cells.	Polymorphonuclear Leucocytes.
1	3·78	18·6	2,845	1,711	13,974
Differ. from Normal.	– 0·72	+ 11·1	+ 1,345	+ 1,211	+ 8,974
Per cent. -	– 13	+ 148	+ 89	+ 242	+ 179

This case is remarkable for the enormous percentage increase in the number of the white cells, and also for the very large increases in the mononuclear cells and leucocytes as compared with those of the other series. Being only an isolated case it is not possible to say whether this is typical of the locality.

Carcinoma of the Larynx.

No.	Red Cells.	White Cells.	Lymphocytes.	Mononuclear Cells.	Polymorphonuclear Leucocytes.
1	2·4	6·3	1,091	819	4,365
—	– 2·1	– 1·2	– 509	+ 319	– 635
Per cent. -	– 46	– 15	– 36	63	– 12

Only one examination was made from this case, and that five days before death ; the lowered number of white cells is difficult to account for, especially as there was extensive ulceration of the growth, and the patient died from septic pneumonia.

SARCOMA.

No.	Red Cells.	White Cells.	Lymphocytes.	Mononuclear Cells.	Polymorphonuclear Leucocytes.
1	4·06	8·7	1,327	868	6,408
2	5·0	8·5	1,816	1,105	5,304
3	3·45	8·7	1,605	825	6,085
4	5·45	17·85	2,030	1,835	13,589
Total - -	17·96	43·75	6,778	4,633	31,381
Average -	4·49	10·93	1,694	1,158	7,847
Normal Average.	4·5	7·5	1,500	500	5,000
Difference -	– ·01	+ 3·43	+ 194	+ 658	+ 2,847
Per cent. -	– 0·2	45	13	131	57

This series shows similar changes from the normal, though to a less degree, especially in the red cells, which have a normal number. Another case, sarcoma of the ischiorectal fossa, contrasts very strikingly with the preceding four :—

No.	Red Cells.	White Cells.	Lymphocytes.	Mononuclear Cells.	Polymorphonuclear Leucocytes.
5	5·8	12·74	2,451	1,931	7,937
Difference -	+ 1·3	+ 5·24	+ 951	+ 1,431	+ 2,937
Per cent. -	28	69	63	286	58

This case is remarkable for the increased percentage of red cells, in spite of the longer duration of the disease, and extensive metastases; of the white cells, the large mononuclears show a greater percentage increase than any of the other averages.

SUMMARY.

1. The preceding tables show that with two exceptions the number of red cells per c.mm. is diminished; and if the exceptional case of carcinoma of the larynx be omitted, there

is in every series an increase of the number of white cells, varying from 37 per cent. to 148 per cent.

2. That with two exceptions there is an increase in absolute number of the lymphocytes per c.mm. varying from 10 per cent. to 107 per cent.

3. That there is in all cases an increase in the absolute number of large mononuclear cells per c.mm. varying from 63 per cent. to 286 per cent.

4. That there is, with one exception, an increase in the absolute number of polymorphonuclear leucocytes per c.mm. varying from 41 per cent. to 179 per cent.

5. That the findings from this review of thirty cases of malignant disease in the human subject are similar in type to those which were obtained from the examination of the blood of cancer mice.

NOTES ON THE BIURET REACTION FOR PROTEINS.

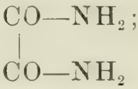
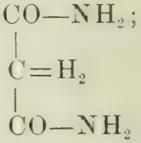
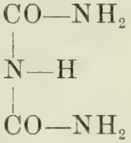
By HECTOR A. COLWELL.

RESEARCHES upon the presence of various enzymes in cancer have rendered it necessary to apply the biuret reaction for proteins under a variety of different circumstances. The following notes upon the subject may therefore be useful:—

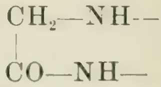
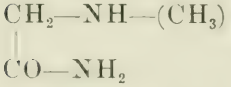
When solutions of a copper salt and of caustic alkali are added to one of a protein, a coloration results which in the case of a native protein is violet, and in that of an albumose or peptone, rose pink. This phenomenon is generally known as the "biuret reaction." A proposal was made by Pickering (1) to designate these two colour developments by the names "ionoproteic" and "rhodoproteic" respectively; it did not, however, meet with general acceptance, and the term "biuret reaction" holds the field for both classes of colour change. This name is, however, not inappropriate, since the reaction is only given by substances which contain certain well-defined chemical "groups"; the structure of the substance "biuret" affords a typical example of one of these groupings, and it was, moreover, the first simple chemical compound to be investigated in this connection.

The first observations upon this subject are due to Rose,(2) who in 1833 investigated the properties of certain metallic albuminates, among which was that produced by the addition of copper sulphate to serum. The precipitate so obtained was, he noticed, soluble in caustic potash with the production of a violet solution. The matter does not appear to have been carried any further as a test for protein. Bence-Jones,(3) however, in 1845, examined the albuminous urine of a patient suffering from osteo-malacia, and noticed that, in addition to its giving a "deep blue" coloration upon addition of $CuSO_4$ and KOH, the blue colour changed to red upon heating.

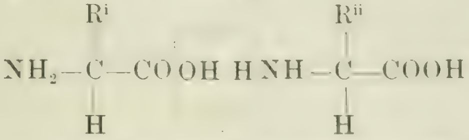
Wideemann,(4) in 1849, noticed that biuret, upon treatment with the same reagents, gave in the cold a rose-pink colour. It is to Piotrowski(5) apparently that we owe the suggestion that the coloration consequent upon addition of $CuSO_4$ and KOH should be used as a test for proteins, and the "biuret reaction" is frequently called by his name.

The latest work upon the subject is that of Hugo Schiff, who has made exhaustive researches into the chemical relationships of the reacting bodies. His results may be briefly summarised as follows:—The biuret reaction is given by various bodies which contain two CO—NH groups, linked in one of three ways: (*a*) the carbon atoms may be directly united as in oxamide  $\begin{array}{l} \mathrm{CO{-}NH_2} \\ | \\ \mathrm{CO{-}NH_2} \end{array}$; (*b*) they may be united by means of a third carbon atom, as in malonamide  $\begin{array}{l} \mathrm{CO{-}NH_2} \\ | \\ \mathrm{C{=}H_2} \\ | \\ \mathrm{CO{-}NH_2} \end{array}$; or (*c*) by the intervention of a nitrogen atom, as in the case of biuret  $\begin{array}{l} \mathrm{CO{-}NH_2} \\ | \\ \mathrm{N{-}H} \\ | \\ \mathrm{CO{-}NH_2} \end{array}$

The above form the three main types of group, but the oxygen atom of the CO—NH groups may be replaced, according to Schiff, by (*a*) a sulphur atom, (*b*) an imino group —NH—, and (*c*) under some conditions by two hydrogen atoms.

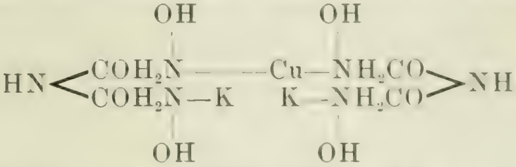
In this last case a group of the type  $\begin{array}{l} \mathrm{CH_2{-}NH{-}} \\ | \\ \mathrm{CO{-}NH{-}} \end{array}$ fulfils the required condition, and is, in fact, found in sarcosine amide  $\begin{array}{l} \mathrm{CH_2{-}NH{-}(CH_3)} \\ | \\ \mathrm{CO{-}NH_2} \end{array}$ which gives a powerful biuret reaction.

This last type of linkage is of especial interest from the point of view of the proteins, since these bodies may be regarded as formed by the union of various α-amino acids, and these by their combination give rise to groups of the type $\mathrm{NH_2{-}\underset{|}{\overset{|}{C}}{-}CO(NH){-}\overset{|}{C}{=}}$, which is similar to that found in sarcosine. That such a group is formed from the combination of two α-monoamino acids, $\mathrm{R^i.CH.NH_2.COOH}$, and $\mathrm{R^{ii}.CH.NH_2.COOH}$ is readily seen,



$$\mathrm{NH_2{-}\underset{\displaystyle H}{\underset{|}{\overset{\displaystyle R^i}{C}}}{-}COOH\quad HNH{-}\underset{\displaystyle H}{\underset{|}{\overset{\displaystyle R^{ii}}{\overset{|}{C}}}}{-}COOH}$$

Schiff considers the formula of the compound produced by the interaction of copper salt, alkali, and biuret to be



$$\mathrm{HN\!<\!\begin{array}{l}\overset{\displaystyle OH}{\overset{|}{COH_2N}}{-}\;\;{-}\overset{\displaystyle OH}{\overset{|}{Cu{-}NH_2CO}}\\ \underset{\displaystyle OH}{\underset{|}{COH_2N}}{-}K\quad\underset{\displaystyle OH}{\underset{|}{K{-}NH_2CO}}\end{array}\!>\!NH}$$

Now the special importance and value of the biuret test lies in the fact that it is given by almost all proteins, but not by the products of their complete hydrolysis—the amino acids. The facts set forth in the preceding paragraph render the reason of this plain, since disruption of the linkages between the constituent amino acids means elimination of the groups which give the biuret reaction.

The marked difference between this and the other "protein reactions" will now be obvious, since the latter are given equally by amino acids and the proteins of which they are constituents, as well as by still simpler bodies. Thus the Millon and Xanthoproteic reactions merely indicate the presence of a hydroxylated benzene nucleus: the reactions of Adamkiewicz and of Hopkins and Cole similarly merely denote the presence of tryptophane. These reactions are consequently only given by proteins which contain the groups in question; whereas the biuret reaction, owing to the almost universal presence in proteins of the type of group which produces it, is of correspondingly frequent applicability. Not only, however, is the reaction of importance for detecting the presence of proteins, but it is also capable of simple and very accurate application in some cases for their quantitative determination. Especially is this true for albumose solutions before and after ereptic digestion.(7)

In consequence of this generality and accuracy of application further information regarding its limits of applicability appeared desirable, and in consequence the experiments which form the subject of the present note were conducted.

Method of Experiment.—Throughout the series the experiments were conducted in Nessler glasses, as suggested by Vernon. The tints obtained were examined by holding the glasses at an angle over a sheet of white paper, and examining the colour obtained by transmitted light. Good diffused daylight is by far the best for the purpose, and was always used when practicable. For quantitative estimations the delicacy of the method is much improved by the use of a black paper mask, pierced for the eyes (Vernon).(7) In any case, the source of light must be identical in any given batch of experiments.

The Solutions employed.—For general work a "Normal" (4 per cent.) solution of sodium hydrate is employed as the alkali. The copper solution I always employ is a centinormal solution of cupric chloride, prepared as required from a stock N/1 solution. This I prefer to the sulphate for two reasons. In the first place if it be desired to vary the alkali by using a hydrate of Ba, Sr, or Ca, the insolubility of the sulphates of these metals would render the test useless; in the second place, strong solutions of copper sulphate, in my experience, exhibit a much greater tendency to the formation of insoluble hydrates than do corresponding solutions of the chlorides.† This dilute copper solution is very convenient, as it allows of small variations in the amount of copper salt added over relatively considerable alterations in the amount of solution used. Even for qualitative determinations I now always perform the test in a Nessler glass, and run in the dilute copper solution from a pipette.

The amount of Protein Solution employed.—This is a matter of utmost importance, since if an excess of protein be added to a given quantity of $CuCl_2$ and NaOH, the resulting tint may be actually *lighter* than when a smaller amount of protein is used. Taking as a standard the tint produced by adding ·4 c.c. of 2·5 per cent. filtered Witte peptone solution to a mixture of 2 c.c. N/100 $CuCl_2$ and 20 c.c. N/1 NaOH, it will be found that by increasing the amount of peptone solution, to, say, 1-2 c.c., the colour becomes distinctly paler and browner. To one who has had much experience in quantitative determinations by this particular method, the peculiar tint given by an excess of the Witte solution at once suggests that something is wrong. The presence of an excessive amount of egg-white or gelatine causes the reaction to be paler though the solution does not become brownish.

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† This can, however, be prevented in both cases by acidifying the stock solution.

If for any particular reason it be necessary to use a larger amount of protein solution as a control in a particular case, the quantity of copper solution used must also be augmented, and in any given series of tests the amount of protein and of copper which gives the optimum tint must be carefully ascertained. This is readily done by mixing the copper and alkali solutions in the quantities mentioned above, and then adding the protein solution from a calibrated 1 c.c. pipette; by using two Nessler glasses and comparing the depth of tint obtained by each increment of protein solution, the optimum result is easily obtained.

The amount of Copper to be employed.—It can be inferred from the previous section that the amount of copper to be added in obtaining the best result must be graded according to the concentration of the protein solution. An excess of copper may easily mask a reaction when the protein is present in minute quantity, and a deficiency of copper, where the amount of protein is considerable, may lead to so transitory an appearance of the reaction that it escapes recognition. I have, therefore, found it better even for qualitative purposes to commence with 1 or 2 c.c. of the dilute copper solution, adding more if necessary.

Variations in the *amount* of alkali used are unimportant.

The Rate of Development of the Reaction.—Vernon (7) has pointed out that the tint produced by the addition of a protein solution to a mixture of $CuSO_4$ and NaOH requires a very definite time to attain its maximum intensity. In the case of Witte peptone, for example, he found that the maximum depth of tint was only attained after eight minutes, although in one minute 93 per cent. of that maximum had been reached. In the case of the native proteins such as those of egg-white and serum, the point was still more marked. Egg-white took twelve minutes to attain the maximum, while with the proteins of serum this was not attained till after two hours, although 50 per cent. of the maximum was reached in thirty-six seconds.

These facts are of manifest importance when quantitative estimations by this method are in progress. Another highly, and from a chemical point of view even more important fact has been recorded by the same observer; namely, that when protein solution is added to the copper alkali mixture, the tint at first developed is always more *pink* than the maximum tint. This is especially marked in the case of serum proteins, as tints which have not attained 50 per cent. of their maximum are so much more pink than the maximum two-hour control as to be incapable of accurate comparison with it.

In a minor degree the same holds for egg-white and even for Witte peptone. From this fact Vernon drew the important conclusion, that "the first portions split off by the caustic alkali are, as regards their biuret reaction, more comparable to peptone."

Variations in the Alkali employed.—Experiments were tried with different alkalis. Those employed were N/1 solutions of KOH, NaOH, LiOH; saturated solutions of $Ba(OH)_2$, $Sr(OH)_2$ $Ca(OH)_2$; and "·880" NH_4OH.

For each experiment 10 c.c. of the alkali solution and 1 c.c. of N/100 $CuCl_2$ solution were taken.

Their actions on different proteins were ascertained by testing 1 c.c. of 1 per cent. solutions of gelatine, egg-white, and Witte peptone, with each of the different alkali mixtures. In the case of the gelatine and egg-white solutions a definite purple tint was given by all the alkalis *with the exception of ammonia*. The solutions of potassium, sodium, and lithium hydrates were identical in their effects; that of barium hydrate was possibly a trifle, and those of strontium and calcium hydrates appreciably slower in development of tint. In the case of the hydrates of strontium and calcium, the pink tint previously described as being prominent immediately after proteins are added to the alkali mixture, certainly persisted longer than in the other cases. The tendency to formation of insoluble carbonates of Ba, Sr, and Ca, however, rendered quantitative determinations impossible. This action upon the part of the two feeblest alkalis under consideration affords interesting confirmation of Vernon's statement concerning the more ready splitting off of the "peptone-reacting" portions of the protein molecule.

Additional confirmation is afforded by the following experiment, in which the concentration of the sodium hydrate solution employed was varied. The quantities taken were 10 c.c. of the alkaline solution, 1 c.c. of 1 per cent. protein solution, and 1 c.c. of N/100 $CuCl_2$ solution. Using N/1 or N/5 solutions of NaOH, the colour was developed with apparently equal rapidity in each case; with N/25 solution it took longer, and with N/125 solution about sixty minutes to attain the same tint. Using N/300 solution, however, no change was noticed, but on leaving the test glass to stand overnight a faint pinkish tint was noted. This rapidly developed into the characteristic violet on addition of concentrated soda solution.

The Action of Ammonium Hydrate.—Thus far the actions of the hydrates of the "alkali metals," and of the metals of the "alkaline earths" have been considered: the question of ammonium hydrate remains. The quantities of the reacting bodies taken were as before stated, 1 c.c. of 1 per cent. protein solution, 1 c.c. of N/100 $CuCl_2$ and 10 c.c. ammonium hydrate (·880). In this case egg-white and gelatine produced no effect, beyond the fact that their addition caused a diminution in the faint blue resulting from the mixture of copper salt, and ammonia; indeed, if sufficient protein were added this would be made to disappear altogether. This is shown in the following actual experiment:—

(1) 25 c.c. $NH_4.OH$ + ·5 c.c. N/100 $CuCl_2$ + 1 c.c. H_2O
= Blue colour.

(2) 25 c.c. $NH_4.OH$ + ·5 c.c. N/100 $CuCl_2$ + 1 c.c.
1 per cent. Gelatine = same as above.

(3) 25 c.c. $NH_4.OH$ + ·5 c.c. N/100 $CuCl_2$ + 1 c.c.
5 per cent. Gelatine = paler.
(4) 25 c.c. $NH_4.OH$ + ·5 c.c. N/100 $CuCl_2$ + 1 c.c.
10 per cent. Gelatine = colour discharged.

With the Witte peptone the result was different; here a definite *pink* tint was obtained, almost identical with that which resulted when the other alkalis were used. On further addition of copper solution, however, the colour changed to violet. The change is of course easily explicable, inasmuch as the excess of copper produces a deep blue with the ammonia, and this combined with the pink tint resulting from their action on the Witte peptone produces a general effect of violet.

This is apparently the reason why Gnezda (8) describes the tint produced by the action of NH_4OH and $CuSO_4$ upon peptone and albumose as violet. He explicitly states that he generally used a deep blue solution made by dissolving a copper salt in ammonia.

An attempt was next made to separate some of the constituents of the Witte peptone; an equal volume of absolute alcohol was added to a 2 per cent. solution of Witte peptone. The mixture was allowed to stand twenty-four hours, and the resulting precipitate separated by filtration. The filtrate contains β proto- and β deutero-proteose. The precipitate is collected and mixed with water, a portion re-dissolves; this soluble part consists of α proto- and α deutero-proteose. The insoluble residue consists of crude hetero-proteose.(9) On examining these three fractions by the copper-ammonia method, the hetero-proteose alone failed to give a pink colour, the proto- and deutero-proteose mixtures both giving a fine pink. This suggests that the method may be available for distinguishing between different proteoses. Further separation of the proto- and deutero-proteoses can be effected by appropriate treatment with ammonium sulphate. In the present experiments it was not attempted, and is only mentioned here to introduce a caution upon the action of ammonium sulphate in the presence of ammonia.

Ammonium sulphate is an easily dissociable salt, ammonium hydrate on the other hand is but feebly dissociable. If now $(NH_4)_2SO_4$ be present in a solution of $NH_4.OH$, the $(NH_4)_2SO_4$ will dissociate freely, tending to saturate the solution with (NH_4) ions, and as a consequence the $NH_4.OH$ will not be called upon to dissociate. Since the action of $NH_4.OH$ as an alkali depends upon its dissociation into (NH_4) and (OH) ions, it is evident that the presence of $(NH_4)_2SO_4$ or other readily dissociable salts of ammonium will hinder or destroy its power as an alkali. And hence if it be desired to perform tests with the copper-ammonia modification of the biuret test, care must be taken to see that all $(NH_4)_2SO_4$ is removed by suitable dialysis.

The Effect of the Presence of Bodies other than Proteins in the Fluids to be Tested.—A certain number of the commoner carbohydrates was examined in this connection. They appeared generally to exert little influence upon the tints obtained unless present in large excess. Saccharose, lactose, maltose, dextrose, lævulose, arabinose, and xylose appeared to have no effect upon the colour in the test glass, even when present in quantities nearly equal to those of the protein employed. If present in great excess, however, the tint is first paler, and on further addition of sugar becomes distinctly yellow. Of the sugars mentioned above, this was most marked in the case of lævulose. Commercial dextrine gives this yellow tint when present in much smaller amounts, and therein differs from starch and glycogen, which produce only a very slight effect.

GENERAL CONCLUSIONS.

1. It is always advisable to perform the biuret test in a Nessler glass, and to run in the dilute (N/100) copper solution from a graduated pipette.

2. Excess of protein may completely mask the reaction.

3. The native proteins give the test with the hydrates of barium, calcium, and strontium, but not with ammonia.

4. Certain albumoses give a pink colour if ammonia is used in place of one of the "fixed" alkalis. Hetero-proteose from "Witte peptone" does not react with ammonia to give the reaction.

5. Experiments with hydrates of Ca, Sr, and Ba confirm Vernon's statement, that the first portions split off from a protein by the alkali assimilate to the peptone type.

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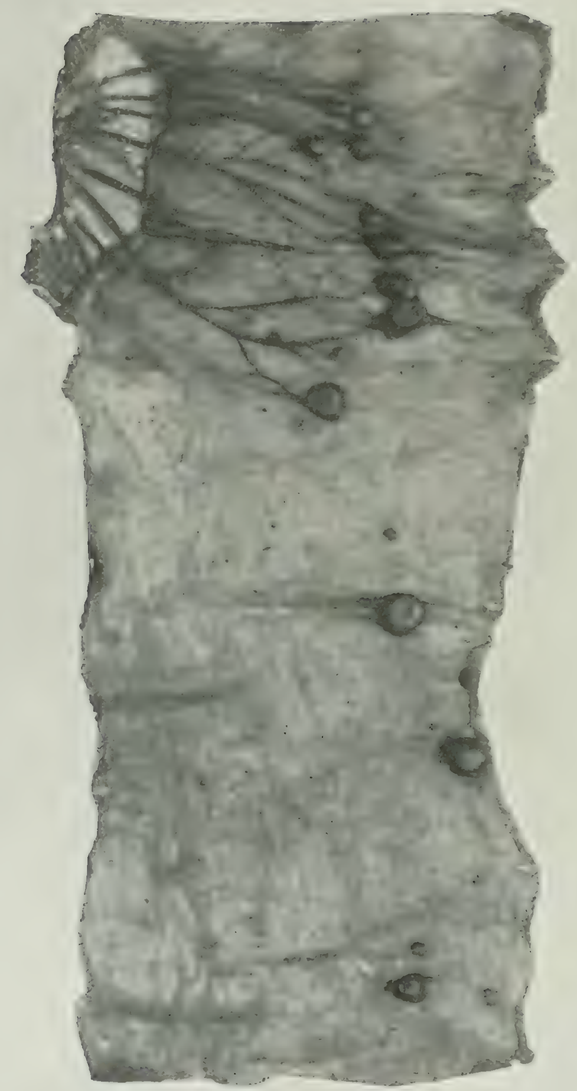

FIG. 1.—Ileum showing multiple tumours (? Endotheliomata).

MULTIPLE INTESTINAL TUMOURS (? ENDOTHELIOMATA) ASSOCIATED WITH LARYNGEAL CARCINOMA.

By B. H. WEDD.

(*With Three Plates.*)

THE interest of the case about to be described lies in the presence of a large number of small tumours in the ileum differing considerably from the growth which caused the patient's death.

The patient, a man aged forty, died in the Cancer Wards of the Middlesex Hospital, having suffered for two years from carcinoma of the larynx. He was admitted to the Hospital with an extensive growth of the larynx and fauces, and at death an ulcerated growth was present, which had destroyed the greater part of the tongue and larynx, involved the jaw, submaxillary glands on both sides, the upper part of the thyroid, and formed an ulcerated mass projecting through the skin in the right submaxillary region. Tracheotomy had been necessary, and broncho-pneumonia was present in the lungs. Nothing else of pathological interest was met with at the autopsy until the small intestine was opened, no metastases being discovered below the level of the upper part of the thyroid.

Small Intestine.—Scattered over the mucous membrane of the middle third of the ileum (i.e., for a distance of about three feet) were sessile polypi, from sixty to eighty in number, and varying in size from that of a millet-seed to that of a pea (Fig. 1). They were irregularly arranged, in places occurring in small groups, with no relation to the mesenteric attachment. They appeared to be somewhat closely associated with the blood vessels running round the gut. They were all submucous, nothing being seen upon the serous surface. The larger ones were umbilicated, but no ulceration could be detected even on microscopical examination.

Histological Examination.

Laryngeal Growth (Figs. 2 and 3).—*Low Power*.—The appearance is that of a typical squamous-celled carcinoma. Masses of growth separated by a small amount of fibrous stroma; no normal tissue present in the section.

High Power (*$\frac{1}{6}$ objective*).—The cells at the centre of the masses of growth are keratinised; cell-nests are present, but not a marked feature. At the edges some differentiation of a Malpighian layer can be recognised. The cell-nuclei vary considerably in size and shape, and some are stained more deeply than others.

Intestinal Growths.—Figs. 4, 5, 6: These are all photographs, taken from a section of a nodule of medium size. Fig. 7: This is a photograph of the muscular coat opposite one of the large umbilicated nodules.

Fig. 4.—The tumour is seen to lie in the submucous tissue, and to be covered with mucous membrane. There is under this magnification a superficial resemblance to the growth in the larynx.

Fig. 5.—The growth is seen at one place to be invading the mucous membrane.

Fig. 6 ($\frac{1}{6}$ objective).—Certain cells have a cubical appearance, and are arranged about a central lumen containing amorphous material; this is specially the case at the edges of the tumour. At the centre there are large masses of cells with nuclei crowded together, and with a small amount of cell-protoplasm. The nuclei are rounded, very regular in size and shape, and with chromatin network clearly stained. Lumina are present in a few of these masses, and small blood-vessels surrounded by the growth.

Fig. 7 shows the growth infiltrating the muscular layer of the intestine.

When the laryngeal and intestinal growths are compared the differences will be seen to be so marked that it seems hardly possible, even allowing for possible variation in character of secondary growths and primary, that these tumours can be so related one to the other. The absence of cell-nests and of keratinisation, the regular and well-stained nuclei, as well as the arrangement of cells about lumina in the in-

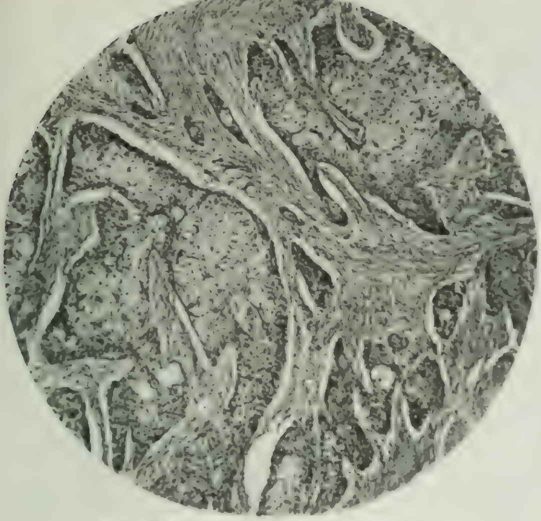

FIG. 2.—Laryngeal growth (low power).

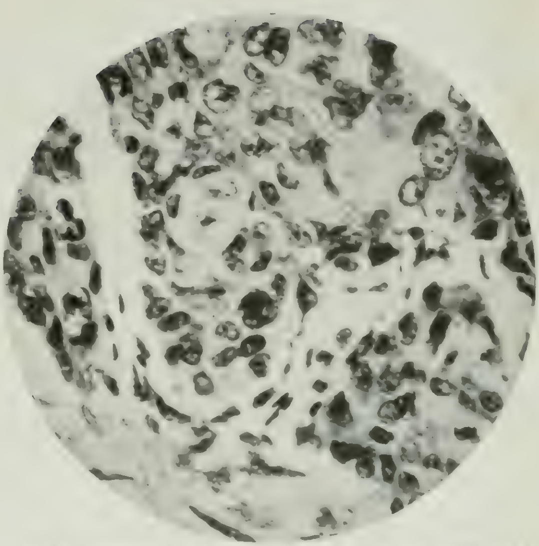

FIG. 3.—Laryngeal growth (high power). (Compare with Fig. 6.)

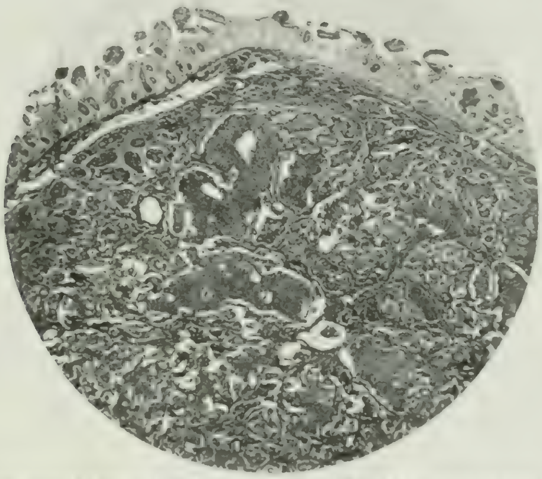

FIG. 5.—Intestinal growth showing invasion of mucous coat at right-hand upper margin (low power).

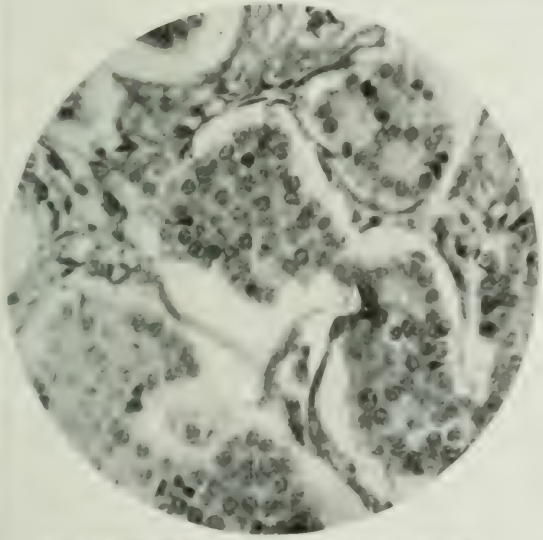

FIG. 6.—Intestinal growth (high power). Note lumina. (Compare with Fig. 3.)

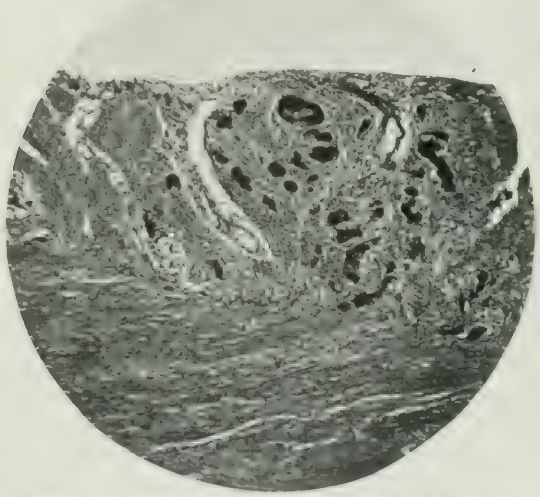

FIG. 7.—Circular and longitudinal muscular coats of intestine showing invasion by intestinal growth.

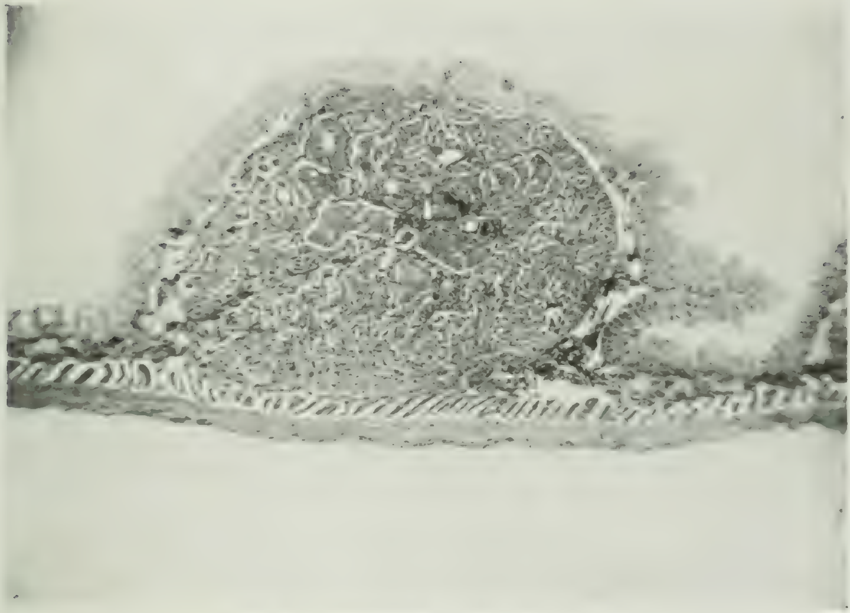

FIG. 4.—Intestinal growth (low power).

testinal tumours, form a picture very different from that obtaining in the growth of the larynx.

Further evidence of their different character was obtained by Beckton's method of fixing and staining for Altmann's granules. Unfortunately the laryngeal growth was not so treated, but Beckton (1) has found that granules are invariably absent from squamous-celled carcinoma. The intestinal growths showed granules irregularly; in many of the cells they were present in fair numbers, in others almost entirely absent. As the tissue was obtained after death the absence of granules from certain cells is of no value as evidence with respect to the malignant character of the tumours, but their presence is good evidence that they are not metastases from a squamous carcinoma.

With respect to the relation of the intestinal growths one to another, there seems no reason to regard any particular mass as a primary focus. There is considerable variation in size, but at no place any specially large tumour, or aggregation of tumours, which could be regarded as a centre of spread. Apart from variation in size, there is no evidence to point to a difference in age of the tumours, and there is very little degeneration and no ulceration in any of the tumours examined to indicate a longer existence. The small size of many of them suggests a recent origin, and in this connection the observations of Hastings, Woodman, and MacCormac (2) upon the rapid increase in numbers of secondary metastases in the later stages of malignant disease is of interest, and suggests the possibility that a recent increase in numbers may have occurred under the influence of the condition produced by the laryngeal growth.

Nature of the Growths in the Intestine.—Polypi of the small intestine of any kind are uncommon; multiple polypi seem to be very rare. Rolleston (3) showed a case before the Pathological Society of multiple polypi of the small intestine which in appearance seem to have closely resembled those in the present case. They appeared to originate in the submucous tissue, and occurred in conjunction with a widely disseminated melanotic sarcoma of the orbit. Histologically they proved to be spindle-celled sarcoma. Kanthack, (4) before the same society, showed a specimen of multiple small polypi obtained from a girl who had died of septicaemia : these proved to be due to a localised hypertrophy of the mucous membrane. Multiple simple polypi of the large intestine have also been described.

The multiplicity of the tumours in the present case suggests either that they are of a simple nature, or secondary to a malignant growth. Evidence that they are not of the same nature as the growth in the larynx has already been adduced. Their histological appearance is not that of the common type of tubular adenoma of the intestine, and though the cells are arranged in places in a manner suggestive of glandular tissue, the cells do not resemble those of the mucous glands of the intestine. The smallest tumour of which sections were made appeared to originate in the submucous tissue, but no lumina were present in this tumour, and there was no evidence of glands in the submucous tissue, such as are present in the duodenum.

There seems to be a resemblance between these tumours and some of the mammary tumours of the mouse, generally regarded as adeno-carcinoma, as well as to certain tumours of the human breast, and from the histological appearances a diagnosis of endothelioma seems justifiable. The appearances of the cells and cell-nuclei correspond with those of tumours of this type, and the presence of lumina has also been regarded as characteristic of such tumours. The infiltration of the muscular coat seen in Fig. 7 shows local malignancy, but in the absence of other evidence cannot be considered of great importance.

Summary.

It is difficult from the above considerations to come to a definite conclusion as to the nature of these intestinal growths. There appears sufficient evidence that they are not metastases from the growth of the larynx. That they are multiple primary growths seems probable, and from their histological appearances it seems possible to consider them either as adenomata, having a tendency to irregular proliferation and infiltration of surrounding tissue, or as endotheliomata of a hæmal or lymphatic origin, the latter being perhaps the more probable explanation, as there seems no evidence of the presence of glands in the submucous tissue which could have given rise to such tumours.

In spite of their tendency to infiltrate the muscular tissue in places, and in spite of their histological resemblance to some cases of adeno-carcinoma in the mouse, and some cases of malignant mammary neoplasm in man, it is probable that they are non-malignant. Their appearances in respect of Altmann's granules is consistent with this view.

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THE POSTERIOR NERVE-ROOT GANGLIONS IN CASES OF CARCINOMA.

By B. H. WEDD.

THE following is a short account of what are really two separate investigations: one made into the condition of the posterior nerve-root ganglions in a series of cases of carcinoma of the breast in women examined post mortem, the other into that of the root ganglions in mice inoculated with a mouse tumour. These investigations were undertaken in the hope of obtaining evidence of certain pathological changes which have been previously described, and were, in part, suggested by observations of Lenthal Cheatle upon the relation of the distribution and spread of cancer to nerve areas, and by the changes he described as being present in the nerve ganglions in certain cases. From observations made chiefly upon rodent ulcers, (1) he came to the conclusion that there was a definite relation between the spread from the primary focus and the distribution of nerves and trophic areas, and also that the incidence of cancer is due to direct or indirect nervous influence upon the area affected. Without going so far as to formulate a definite theory, he suggested that a connection between preceding chronic irritation and subsequent cancer may be found in changes in the nerve ganglions. In support of this suggestion he described what he believed to be pathological changes in certain ganglions. The first of these was from a woman suffering from carcinoma of the breast, accompanied by agonising pain, to relieve which he removed the fifth dorsal ganglion on the affected side. The ganglion was reported as showing changes in certain cells, such as have been described by Lugaro as the result of chronic irritation. In view of the fact that only one ganglion was examined, and of the difficulty of recognising as pathological slight changes in ganglion cells, much importance cannot be attributed to this statement. Later the same author (2) described two cases of cutaneous cancer, examined post mortem, associated with severe pain. In these the ganglions corresponding to the area of the primary site showed inflammatory changes, not found in adjacent ganglions or in the corresponding ones of the opposite side, although secondary growths were present in the areas of distribution of these nerves. Intracellular degeneration was also present, but not confined to the particular ganglions showing inflammatory changes.

In the hope of obtaining further evidence of changes in the posterior nerve-root ganglions, five cases of carcinoma of the breast which died in the cancer wards of the Middlesex Hospital were examined. No attempt was made to obtain the ganglia immediately after death, the shortest interval between death and autopsy being sixteen hours. It was recognised that under these circumstances a comparison of the corresponding ganglions on the two sides was essential.

Method Employed.—The second, third, fourth, and fifth dorsal ganglions were removed, fixed by Lenhossek's method, embedded in paraffin, and the sections stained by various methods, of which Weigert's haematoxylin and Van Gieson seem most satisfactory. Nissl's methylene blue method and Leishman's stain were also used to show cell granules.

The ganglions from the two sides were compared, with respect to the thickness of the capsule, the presence of inflammatory changes in the ground substance, and the condition of the nerve cells. No differences were made out either in capsule or ground substance upon the two sides in any case. More difficulty was experienced in comparing the condition of the ganglion cells. On both sides many cells were seen in which the Nissl's granules were fine and dust-like ; a few cells were seen with the nucleus placed eccentrically, and vacuoles were also seen in a proportion, but these changes were not more marked on the affected side, with the exception of one ganglion in a single case. In this instance the third dorsal ganglion on the affected side showed very marked chromatolysis as compared with the corresponding one ; this condition was confined to one side of the ganglion, and was not noticed in the other cases examined, or in the other ganglions from the same case. The skin in this case was extensively ulcerated, and adherent to the ribs, which were atrophied and very thin. Possibly the condition of the chest wall and of the ganglion are associated, but in the absence of any evidence from the other cases, this cannot be regarded as certain.

An investigation was also made into the condition of the posterior root ganglions in mice inoculated with cancer. It was thought that changes secondary to the growths might be recognisable, although, in the case of inoculated tumours, antecedent changes could not be expected.

Method.—Mice were selected with large tumours, covering the greater part of one side of the thorax. An effort was first made to remove the dorsal ganglions singly, and to examine them in pairs. Numerous ganglions were so removed, but much difficulty was found in obtaining a complete series from one mouse. These were treated in a similar way to the human ganglions. No differences could be recognised on the two sides. Some of these were stained whole in a dilute solution of Ehrlich's methylene blue (for intra-vitam staining) in normal saline, but owing to the fatty capsule penetration was not good, and the results unsatisfactory. Finally the whole of the spinal column in the dorsal region was removed, decalcified, and sections made longitudinally. By this method sections were obtained of the ganglions of both sides, but no differences could be discovered

Under the conditions under which these investigations were made, that is, upon post-mortem material obtained some time after death, no evidence was obtained of changes either primary or secondary in cases of carcinoma of the human breast. The ganglions of mice inoculated with cancer appear normal.

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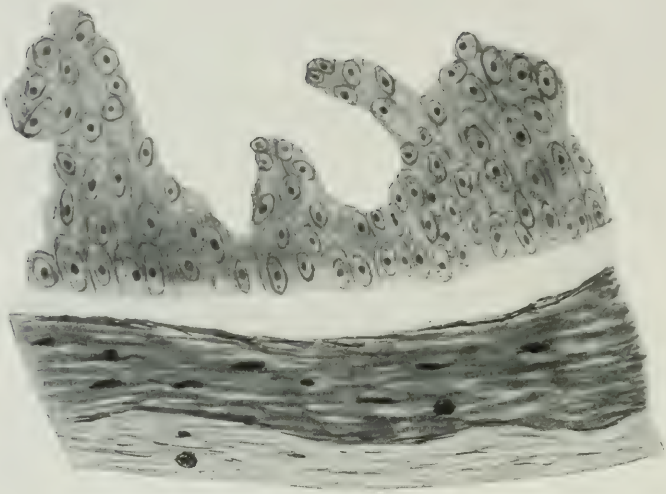

FIG. 1.—Columnar-cell growth (high power).

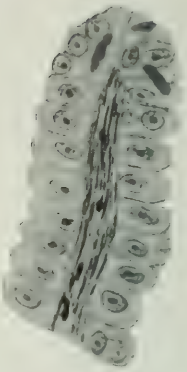

FIG. 2.—Columnar-cell growth
A villus (high power).

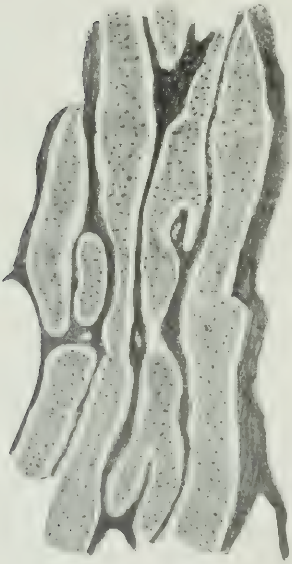

FIG. 3.—Spheroidal-cell growth
(low power).

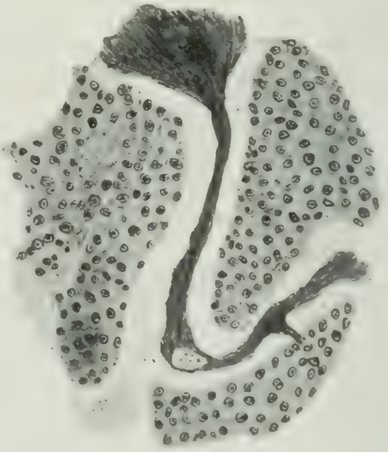

FIG. 4.—Spheroidal-cell growth
(high power).

DOUBLE PRIMARY GROWTH IN ONE BREAST.

A CASE OF DOUBLE PRIMARY GROWTH IN ONE BREAST.

By M. BENMOSCHE.

(*With Plate.*)

Cases of double primary malignant growths have been reported from time to time, although it is quite probable that their occurrence is more common than the literature on the subject would lead us to think. R. A. Young reports several cases which have occurred in the Middlesex Hospital. ("Archives of Middlesex Hosp." vol. 3.) In this report he also quotes Billroth's Criteria for the establishment of a diagnosis of double primary malignant growths, published in 1889:—

(*a*) The growths must be of different histological structure.

(*b*) Each growth must take its origin in normal epithelium of its own type.

(*c*) Each growth must produce metastases histologically similar to itself.

M. L. Bard, in 1892, regarded the last of these criteria as not absolutely essential, and this view is generally accepted.

The present case (No. $\frac{254}{07}$) is that of a woman aged 74 who died in the Middlesex Hospital in 1907. In 1901 she first noticed a tumour in the breast which grew steadily and ulcerated in 1905. The growth was freely movable on the chest wall. Death was the result of gangrene of the leg. At the autopsy the upper and outer quadrant of the right breast showed an ulcerating growth penetrating the skin, almost circular and measuring about two inches across. It projected from the level of the skin and the nipple was left intact. No metastatic growth was found in the body.

Microscopic sections show two distinct kinds of new growths; one of a low cylindrical or columnar cell type, and the other of a more or less spheroidal cell type.

To facilitate description the sections will be referred to as No. 1 (Figs. 1 and 2) and No. 2 (Figs. 3 and 4) respectively.

In No. 1 the glandular structure of the breast is principally involved. The acini and ducts are dilated to an extent that characterises them as cystic. These cysts are formed by a dense fibrous connective tissue stroma, enclosing several layers of epithelial cells more or less retracted from the surrounding walls. The fibrous framework here and there enters the cysts, forming the supporting element of papillomatous projections or distinct villi covered over by tall, or in places low, columnar cells. These cells have large, clear and prominent nuclei, the majority of which show very small nucleoli and fine points of chromatin marginally arranged. The cytoplasm is clear, well-outlined, and very faintly granulated. Mitotic characters are scarce.

The fibrous matrix, immediately surrounding the cellular areas, is infiltrated with aggregations of closely packed lymphocytes and an occasional plasma cell.

The adipose tissue, throughout, is markedly infiltrated with cells, which present the identical characteristics of those concerned in the tumour proper. The lymphocytes are few and widely scattered.

No. 2 is derived from that part of the growth which was found immediately below, and ulcerated through, the skin surface. The loose connective tissue of the dermis is infiltrated with a large number of scattered lymphocytes, plasma cells, and numerous large spheroidal cells with vesicular nuclei. The growth appears as a distinctly lobulated, spheroidal-cell mass, the boundaries of which are formed by a dense connective tissue framework infiltrated with occasional lymphocytes.

The character of the cell is different from that of No. 1. It is more or less of a spheroidal type. Its cytoplasm is granular, and although many of the nuclei appear clear with small nucleoli and delicate points of chromatin, most of them stain much more intensely with hæmatoxylin than do those of No. 1, and a great many show mitotic changes.

Neither growth having given rise to perceptible metastasis, the malignant character of either can only be judged by its histological appearances, but they conform well to the types of villous columnar-cell carcinoma and spheroidal-cell carcinoma respectively.

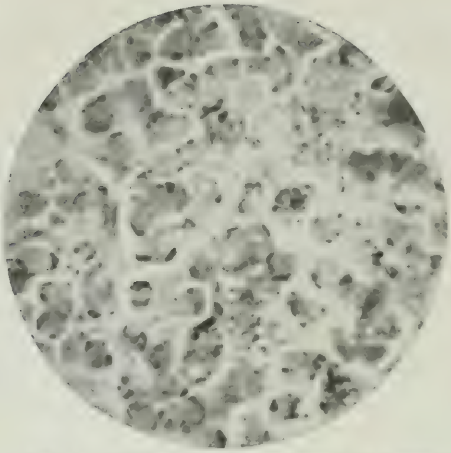

FIG. 1.—Section of spleen exposed in a small volume of air for 24 hours. Note the deep black foci of granule accumulation at the margins of the cells.

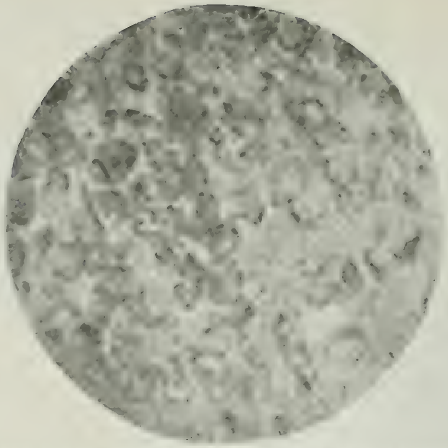

FIG. 2.—Section of spleen exposed in a small volume of radium emanation for 24 hours. Note the almost complete disappearance of granules from the cells.

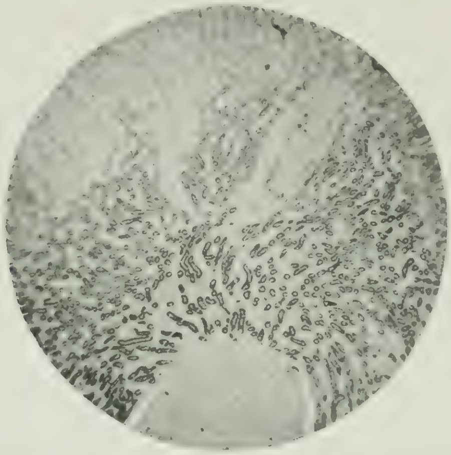

FIG. 7.—Section of kidney exposed in a small volume of radium emanation for 24 hours. At the lower part of the field is seen the agranular pyramid; at the upper part the outer zone of the cortex from which the granules have disappeared. Between these is the inner zone of the cortex showing granules in the cells of the tubules when examined under a high power.

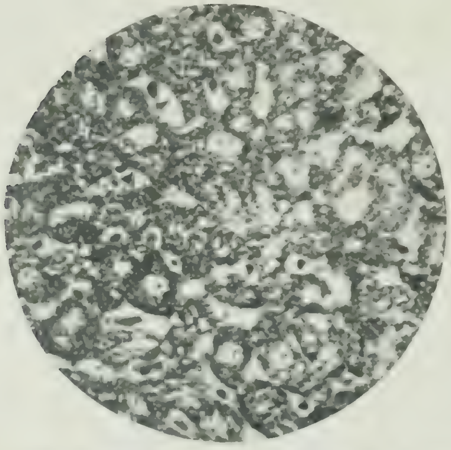

FIG. 5.—Section of liver exposed in a small volume of air for 24 hours. The liver cells are crowded with granules, thus giving a sharp black appearance. (Note that nucleoli and red blood cells have also retained the stain.)

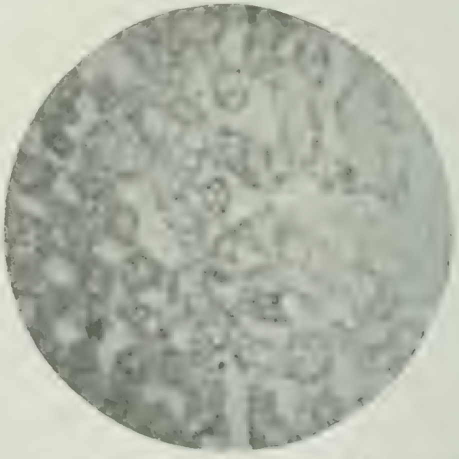

FIG. 6.—Section of liver exposed in a small volume of radium emanation for 24 hours. The granules of the liver cells have almost all disappeared. (The same is true of nucleoli and red blood cells.)

The sections from which Figs. 5 and 6 were photographed were stained and differentiated simultaneously on the same slide.

THE EFFECT OF RADIUM EMANATION ON ALTMANN'S GRANULES.

By H. BECKTON and S. RUSS (Beit Memorial Research Fellow).

(*With Plate.*)

It has been established by one of us[†] that there is a marked absence of Altmann's granules from the essential cells of malignant new growths as contrasted with their presence in the corresponding species of normal cells. To determine the action of the different radiations from radio-active substances upon these granules, experiments have been made in which specimens of freshly excised normal tissues have been exposed *in vitro* under various conditions to the α, β, and γ rays which are emitted by radium emanation and its short-lived products Ra.A, Ra.B and Ra.C.

Spleen.—A slice of mouse's spleen about 1 mm. thick was placed in a small test tube (diam. about 7 mm.); the tube was filled with mercury and inverted in a small trough, and radium emanation, mixed with about ·75 c.c. of air, was introduced into the tube. The emanation was equal in amount[‡] to the equilibrium value of 9·02 mgms. of $RaBr_2$, as measured by its γ ray activity, three hours after its introduction into the tube. Subsequent experiments to be described were made with about this order of concentration of the emanation.

The exposure of the spleen to the emanation lasted twenty-four hours. On the following days the tissue was fixed in formol-Müller (1-49), embedded and stained for Altmann's granules as described elsewhere.[§]

It was found that these granules were absent from the lymphoid tissue of which the spleen is largely composed. (*Vide* Figs. 1 and 2 on Plate.)

[†] H. Beckton, "Arch. Midd. Hosp.," 8th and 9th Cancer Reports.

[‡] Such quantities will elsewhere be referred to, for brevity, as so much emanation.

[§] Loc. cit.

Two controls were provided in the above experiment. In one a slice of the same spleen was exposed over mercury to air only, and in the other a slice was placed directly in formol-Müller. In both of these controls Altmann's granules were abundant in the lymphoid tissue.

This experiment was repeated on several occasions with amounts of emanation varying from 3·1 to 9 mgms., with practically identical results.

In view of the fact that when the emanation is pumped off radium salts it usually contains gaseous impurities, a series of exposures of spleen in hydrogen, carbon dioxide, and coal gas was made. Subsequent examination of the specimens of spleen showed that none of these gases was the cause of the previously described disappearance of granules.

The radiation from the emanation and its short-lived products being complex, it was desirable to determine which type of radiation was responsible for the disappearance of granules.

The effect of excluding the α rays was examined in the following manner:—A small glass bulb, thick enough to prevent the emergence of the α rays, but allowing free exit of the β and γ rays, was filled with 19·3 mgms. of emanation, and

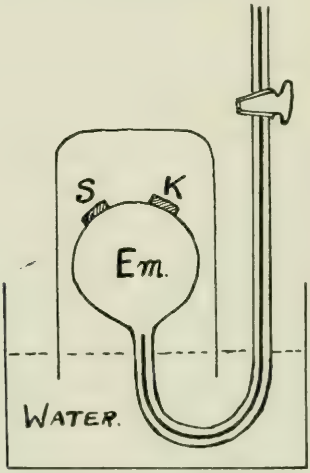

FIG. 3.—Diagram showing pieces of spleen (S) and kidney (K) exposed in a small volume of (moist) air to the action of the radiations passing through a small thin glass bulb filled with radium emanation (Em.).

a thin slice of freshly excised mouse's spleen was placed on the outer surface. This system was confined in a small saturated enclosure as indicated in Fig. 3. The intensity of the β and γ radiations to which the specimen of spleen was subjected was practically the same as in the experiment initially described.

After an exposure of 20 hours, followed by the usual fixing and staining process, examination for Altmann's granules showed that these were abundantly present in the lymphoid tissue.

This experiment indicated that the disappearance of Altmann's granules from the lymphoid tissue of spleen exposed to the emanation was due to the action of the α particles.

Bearing in mind the very small penetration of matter by these particles[†] and the disappearance of the granules throughout the entire thickness of the specimen of the spleen, it was clear that the emanation diffused into the tissue. The extent to which it does so was found in the following manner:—A piece of mouse spleen was exposed to the emanation for one day; it was then removed, mounted upon a freezing microtome, and thin slices trimmed off all its surfaces. The inner portion was then sealed in a glass tube, and subsequently examined for emanation. The concentration of the emanation in the tube at the end of the exposure was 7·1 mgms. per c.cm., and in the portion of the spleen examined it was 3·85 mgms. per c.cm., thus giving a "coefficient of absorption" of 3·85/7·1 ·54.

The disappearance of Altmann's granules from lymphoid tissue when exposed to the emanation is therefore due to the α particles ejected by that portion of the emanation which is absorbed by the tissue. The nature of this action, however, is not evident. It is at present unknown whether Altmann's granules exist as such, i.e., as small, discrete, sharply-defined bodies, in living or freshly-excised lymphoid tissue, or whether they are formed from some "granule substance" as a result of the various processes to which a specimen of tissue is subjected in order that it may exhibit these granules.

[†] The maximum range in this case is that of the α particle from RaC, which is 7·06 c.ms. of air at 76 c.ms. pressure. Assuming the density law to hold, this is equivalent to a distance of ·03 mm. water, which may without much error be taken as the range in the tissue here dealt with.

With a view to obtaining some information on this point, as well as confirmation of the results already arrived at, a specimen of mouse's spleen was radiated by α particles, without, however, the emanation coming into contact with the specimen, and consequently becoming distributed throughout it. This was done in the following manner:—

Two slices of spleen, S_1, S_2 (Fig. 4), about 2 mms. thick, were placed on a thin sheet of mica, each surrounded by a rim of vaseline and covered with another sheet of mica. The mica sheets were thin enough to let α particles through with little reduction in their range.

One specimen was subjected to α rays on its top and bottom surfaces, as indicated in Fig. 4, the other being protected from this radiation:—

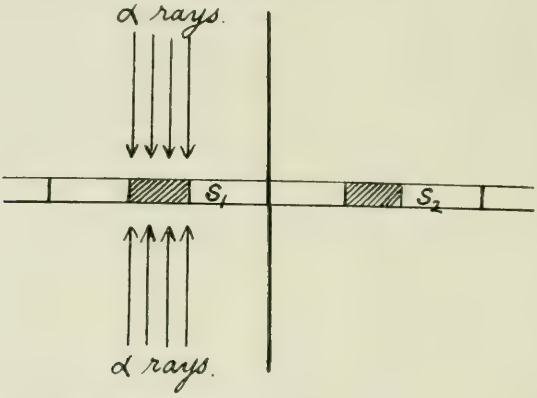

FIG. 4.—Diagram indicating a piece of spleen (S) exposed on both sides to the action of radium bromide. This, contained in two applicators, is separated from the spleen above and below only by two thin films of mica and a small thickness of air.

Under the circumstances of the experiment, disappearance of the granules would not be anticipated at a depth from the surface greater than the range of the α particles, unless under the action of these particles the substance of which the granules are composed is broken down with the formation of fluid which diffuses out of the cells into the surrounding tissue, and there destroys other granules.

After an exposure of 24 hours, examination of the lymphoid tissue for Altmann's granules showed that, while in the control they were present right up to the edges of the section, in the radiated specimen they were almost completely absent to a depth below both surfaces just less than the calculated range of the a particles, the line of demarcation of the granular and non-granular cells being well defined. Even in these zones of disappearance of granules, however, cells were to be seen here and there showing a few granules, a fact somewhat difficult to reconcile with the supposition that the granules are formed from some fluid pervading the cell-substance, but which might reasonably be anticipated if the granules were initially discrete.

This experiment confirms the conclusion that the effective agent in the disappearance of granules is the a particle, the action appearing to be restricted in that the granules are not affected beyond the range of the a particles. Had any diffusion of radiated "granule substance" occurred, with subsequent action upon other cells, to a distance of ·01 mm., it could have been detected.

On staining with hæmatoxylin and eosin, the radiated specimen was found to lack nuclear detail in the zones from which the granules had disappeared. This phenomenon, due apparently to a diffusion of chromatin throughout the nucleus, had already been noted in tissue exposed to the direct action of radium emanation.

Liver.—Thin slices of this organ from the mouse were exposed simultaneously with the spleen to the direct action of emanation. A marked disappearance of granules in the radiated specimen was again observed. Figs. 5 and 6 (see Plate) are microphotographs representing respectively the air control, which shows an abundance of granules, and the radiated specimen, from which the granules have almost entirely disappeared.

Pancreas and Duodenum.—The effect of the emanation upon the spheroidal secreting cells of pancreas and the columnar secreting cells of duodenum was determined by the method already detailed.

In the case of pancreas, but not in the case of duodenum, exposure of a specimen to air for twenty-four hours, followed by the usual fixing and staining, shows complete disorganisation of the tissue, and under these circumstances Altmann's granules are absent from both pancreas and duodenum. The disorganisation of tissue referred to is usually attributed to the action of digestive ferments.

When pancreas and duodenum were exposed to the emanation for twenty-four hours, histological detail was preserved in the two specimens, but Altmann's granules were absent from the pancreas and present in the duodenum, though staining diffusely.

In this connection, reference may be made to the work of Henri and Meyer,† who showed that the trypsin and amylopsin in pancreatic juice are rendered inactive when exposed for considerable periods to the radiations from radium. The type of radiation producing this effect is, however, not stated; the quantity of radium used was large, being 100 mgms.

The opportunity has not presented itself of exposing the active ferments of the pancreas to quantities of emanation such as were at our disposal in the experiments upon the tissues just described. Liq. pancreatis, which was exposed for several days to the α, β and γ radiation from 2–3 mgms. of $RaBr_2$, however, showed no appreciable diminution in its activity when tested by its action on starch mucilage and gelatine. We are, therefore, unable to say whether the preservation of the histological detail of pancreas in the presence of the emanation is due to an inhibitory action upon the ferments which otherwise account for its destruction.

Kidney.—A thin slice of this organ from the mouse, fixed directly in formol-Müller, shows absence of Altmann's granules from the pyramid, but presence throughout the cortex.

After exposure of a specimen to emanation for twenty-four hours in the manner already described for the spleen, it was found that three zones were clearly differentiated, as seen in Fig. 7 (see Plate), viz. (1) pyramid, agranular; (2) an intermediate zone in which the cells of the tubules were closely packed with large granules; (3) an outer zone, agranular. In this outer zone, however, a few isolated tubules were observed, similar in appearance to those in the intermediate zone. An air control showed absence of the granules from the pyramid (1), but presence throughout the cortex (2) and (3).

A chemical difference is thus suggested between the granules of the inner and outer zones of the cortex. The sharpness of separation of zones (2) and (3) renders it very improbable that the effect is due to some variation in concentration of the emanation in the two portions.

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† Henri and Mayer, Comptes Rendus, 1904.

Continued exposure to the emanation, however, results in the disappearance of the granules from the intermediate zone (2). A specimen of kidney exposed to the emanation, in the manner indicated above, for three days showed an almost entire absence of granules throughout; the air control in this case showed a nearly complete disappearance of granules in the outer zone (3), a moderate number remaining in the intermediate zone (2).

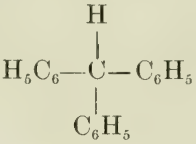
Elimination of the α rays from the complex radiation from the emanation showed, by the non-disappearance of the granules, that here again the α particle is the effective agent. This was done in the manner already described for the spleen, K in Fig. (3) being the specimen of kidney.

These results indicate the presence in the cortex of two types of granules, which not only manifest differences under the action of the α particle radiation, but also exhibit varying powers of resistance to the autolytic ferments present in this organ.

A NOTE ON STAINS FOR ALTMANN'S GRANULES.

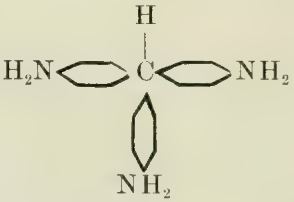
By the late THERESA DE G. MILLER,
M.B., B.S.(Durh.).

The experiments recorded in the present note were undertaken with the object of throwing some light upon the chemical constitution of Altmann's granules. These bodies stain with acid fuchsin, and accordingly a series of other acid dyes of allied chemical structure was made the subject of experiment. The stains selected were acid cyanin, acid violet, "Säuregrün," "Lichtgrün," patent blue, thionin, and cyanin. These in common with acid fuchsin may be regarded as derivatives of triphenylmethane—



$$H_5C_6—\overset{\displaystyle H}{\overset{|}{\underset{\underset{\displaystyle C_6H_5}{|}}{C}}}—C_6H_5$$

If, in each of the phenyl groups, that hydrogen atom which occupies the paraposition relative to the methane carbon atom is replaced by an amidogen radicle, a body known as paraleucanilin, or *p.* triaminotriphenylmethane results—



This is the mother substance of all the dyes under consideration. A further point of agreement in their chemical

Note.—This paper has a pathetic interest. The writer passed away on June 12th, at the moment when the printed evidence of work she had so cheerfully done in the laboratories was arriving. Cancer was a subject of absorbing interest to Miss Miller, and her subordination of self and of poor health to the desire of adding an iota to our knowledge for the benefit of others was a lesson in altruism that will long bear fruit in the place where she was so welcome a colleague.

relations is the presence of a sulphonic acid (HSO_3) residue in one or more of the benzene nuclei, and it is upon the presence of the hydrion in this side chain that their colour depends, since upon complete neutralisation the resulting compound is colourless.

The acid fuchsin used for the demonstration of Altmann's granules is dissolved in anilin water. Anilin belongs to the class of bodies termed by Gustav Mann "accentuators." The precise mode of action is unknown, but their presence allows of combinations between the tissue and the stain which would otherwise be difficult or impossible.

Three of the dyes, namely patent blue, thionin, and cyanin, gave discouraging results. Patent blue assumed an insoluble, india-rubber like consistency on the addition of anilin water, thionin dissolved with great difficulty and deposited numberless red crystals on the sections, whilst cyanin was insoluble either in anilin water or in distilled water.

The original solution of acid fuchsin is as follows:—

Acid fuchsin...	·5 gm.
Anilin water...	2·5 c.c.

but the remaining four stains showed such a tendency to adhere in a sticky insoluble mass, when mixed with anilin water, that the solution was modified thus:—

Stain	·5 gm.
Distilled water	5·0 c.c.
Anilin water	2·5 c.c.

The sections were stained at 60° C. for varying lengths of time, and subsequently, as in the acid fuchsin method, treated with picric acid alcohol, absolute alcohol, and xylol. The results were poor.

Acid cyanin showed some granules in some, but not in all of the sections, the granules were not evenly distributed, neither were they clearly discrete. Acid violet exhibited similar appearances, only one section showed granules with clearness, in the others there were but few, and those mostly of a large size. Säuregrün gave general staining, with no granules apparent. Lichtgrün, though a few granules were seen in a small number of cells, also stained generally.

Following up a suggestion as to the acid nature of the granules, sections were soaked for one minute before staining in hydrochloric acid of strengths of 100, 50, 25, 12, 6, 3 per cent. respectively. The same stains were used in similar fashion. Those in 25 per cent. acid showed some slight indications of granular staining, but the granules were large, and in two sections only did any suggestion of granules in lymphoid tissue appear.

A solution of acid cyanin in hydrochloric acid 25 per cent. was next tried as a stain ; the typical granules did not appear, though large granules were present in secreting cells, and smaller ones in large quantities in duct cells. The smaller ones stained of a bluer tinge than the larger green granules, just as with acid fuchsin some granules are more purple than the small crimson granules.

Fresh tissues were then prepared. Separate portions of mouse kidney were kept for twenty-four hours, at room-temperature, in hydrochloric acid of strengths of 5, 4, 3, 2, 1 and ·5 per cent. respectively. They were transferred into formalin and Müller solution, where they remained for six days, subsequently passing through water, alcohols, and xylol, to paraffin.

The results of using the stains were as follows :—

Acid Fuchsin.

5 per cent. HCl (No. 1). Very deep staining, specially in the cortical portion, granules somewhat swollen in appearance, not clearly discrete, surrounding tissues not well differentiated.

4 per cent. HCl (No. 2). Granules not discrete.

3 per cent. HCl (No. 3). Section less deeply stained, and an appearance of granular protoplasm rather than granules.

2, 1, and ·5 per cent. HCl. Increased in indistinctness.

Acid Cyanin (Red screen used).

5 per cent. HCl (No. 1). Granules apparent, clearer and more discrete than those of acid fuchsin No. 1, and the section as a whole less deeply stained.

4 per cent. HCl. Heavy staining in parts, in others discrete granules.

3 per cent. HCl. This strength of acid gave the best result, general staining less, granules apparent.

2 per cent. HCl. Granules, where discrete, of the large variety only.

1 and ·5 per cent. HCl. No granules.

Lichtgrün (Red screen.)

5 per cent. HCl (No. 1). Large discrete granules appear, and a few small ones. (A better result than when no acid was used.)

4–·5 per cent. HCl. Progressive inferiority to No. 1.

Säuregrun.

5–·5 per cent. HCl. No small granules, a few large in 5 per cent. specimen.

Acid Violet.

5 and 4 per cent. HCl. Much overstaining, suggestion of granules in 4 per cent specimen.

3 and 2 per cent. HCl. Granules do appear, but not very discrete, poor differentiation.

1 and ·5 per cent. HCl. "Faded" appearance recurs as in other sections.

Though the data are not satisfactory, one learns that tissues treated with acid become more retentive of the stains used, that in some cases granules appear more clearly than in the unacidified sections, and that the lowest strengths of acid used were so weak as to exercise the same influence, apparently, upon the granules as does water, so that they do not stain properly even with acid fuchsin.

These staining methods though showing results inferior to those obtained with anilin-acid-fuchsin, nevertheless confirm the view that Altmann's granules are acid in character. The inferiority of the results may be due to at least two obvious causes; in the first place the stains were employed much more dilute than was the case with the acid fuchsin solution; and in the second, anilin may be unsuitable as an accentuator under these particular conditions.

THE ACTION OF RADIUM RADIATIONS UPON SOME OF THE MAIN CONSTITUENTS OF NORMAL BLOOD.

By HELEN CHAMBERS, M.D., and

S. RUSS, D.Sc. (Beit Memorial Research Fellow).

The results of this Enquiry were published in "Proc. Roy. Soc.", ~~June~~ July, 1911.

The following experiments were undertaken with a view to determining the effect *in vitro* of the different radiations from radio-active substances upon some of the main constituents of normal blood.

The observations have so far been extended to the hæmolytic action of the α rays on red corpuscles, to the effect of these rays on leucocytes, and to their action on opsonin and complement.

Numerous experiments have also been made with the β and γ rays, but almost without exception the results have been of a negative character, probably on account of the limited amount of radium which has been used.

Preliminary Experiments on Sheep's Blood.

A small glass bulb of volume 10 c.cs. was provided with two taps; the bulb was evacuated and emanation in equilibrium [†] with 16·7 mgms. $RaBr_2$ let in. About 2 c.cs. of whipped sheep's blood were then allowed to run in through one of the taps, a control being retained. After twenty hours in the ice chest, marked hæmolysis was observed in the blood contained in the emanation bulb, the control being unaffected. The spectrum of the blood, when examined, was found to correspond to that of oxy-hæmoglobin alone. The experiment was repeated with a slightly smaller quantity of emanation with similar results.

[†] Such quantities will subsequently be referred to as so much emanation. The activity was always determined by a comparison of its γ-ray activity with that of a standard.

Hæmolysis and the formation of met-hæmoglobin has been observed by Henri and Mayer [†] experimenting with frog's blood and that of the dog by exposing it to 100 mgms. of radium. The type of radiation producing these results is, however, not stated.

It was decided to see whether the effect was due to the action of the emanation on the corpuscles themselves or upon the serum in which they were present.

For this purpose some whipped blood was centrifugalised, the serum syphoned off and exposed to 6·4 mgms. emanation. The red corpuscles were thoroughly washed with saline and exposed to 9·89 mgms. emanation, the exposure in both cases being for twenty hours. The washed red corpuscles showed marked hæmolysis. The radiated serum, however, when added to some of the original non-radiated red corpuscles showed no hæmolysis after twenty-four hours. This shows that the action on the red cells is probably to be attributed to a direct effect of the radiations from the emanation and its products.

It must, however, be remembered that the gaseous emanation, when pumped off from radium salts in solution, usually contains traces of other gases.

The hæmolysis might then possibly be due to some of these gases or to the emanation acting simply as a gas dissolved in the blood and in some way inducing hæmolysis. The effect of the gases mixed with the emanation was tested by storing the gaseous contents of one of the glass bulbs (containing emanation) for over a month, by which time the emanation had decayed to less than 1 per cent. of its original amount. The gases were then mixed with blood in the usual way. No hæmolysis was visible after three days' exposure.

It will be seen subsequently that the emanation does dissolve in blood, but that the hæmolytic effect can occur quite independently of this solution process.

The Effect of the Radiations on Human Blood.—Hæmolysis occurs in human as in sheep's blood, and it was resolved to see which of the radiations, α, β, or γ was responsible for the effect.

[†] Henri and Mayer. Comptes Rendus, p. 521, 1904.

A very thin glass tube was filled with 9·14 mgms. emanation. The glass was just sufficiently thick to stop all the α rays, but to absorb practically none of the β or γ radiation. This tube was surrounded by another one containing citrated blood and kept for forty-eight hours at 0° C. No hæmolysis was observed.

This experiment has been repeated on two subsequent occasions with 9·76 mgms. emanation and with 20 mgms. of pure $RaBr_2$ sealed in a thin glass tube. In neither of these experiments was hæmolysis observed.

The effects, then, were clearly due either to some action of the emanation acting as a gas or to the α rays emitted by it and its products RaA and RaC.

Assuming, as is subsequently shown to be the case, that the hæmolysis is due to the α particles, and remembering their exceedingly small penetration of matter (7·08 cms. of air being the maximum range of the α particles, in this case from RaC), it was clear that the results obtained were due to the emanation partly dissolving in the fluid which held the corpuscles.

The "coefficient of absorption" of serum was determined in order that some idea might be obtained of the number of particles required to effect the release of the hæmoglobin from the corpuscles.

This was done in the following way: A spherical bulb was half filled with serum (sheep's), the remaining half containing 5·95 mgms. of emanation mixed with air at atmospheric pressure and room temperature. A long interval was allowed in order that equilibrium should obtain between the emanation dissolved in the serum and that in the contained air. The serum was then carefully driven out of the bulb under a fractional increase of pressure, and the reduction in the activity of the emanation remaining in the bulb was then measured. The "coefficient of absorption" obtained in this way was ·18, which shows that a very considerable fraction (between one-fifth and one-sixth) of the emanation goes into solution in the serum. There is, therefore, no difficulty in seeing how the hæmolysis may occur as the result of alpha radiation.

The Gradual Release of Hæmoglobin from the Corpuscles.

When blood was exposed to the emanation in closed glass bulbs an interval elapsed before hæmolysis was visible, and it was decided to watch the process from its earliest stages.

For this purpose a glass bulb of 30 c.cs capacity was filled with 26·5 mgms. of emanation and 2 c.cs. of citrated blood. The bulb was closed by two capillary taps, and a small sample of the blood could be obtained by effecting a small reduction in pressure on the far side of one of the taps.

The earliest observations showed that free hæmoglobin was present before any visible change had occurred in the red corpuscles. The liberation of hæmoglobin, therefore, was a gradual process.

For this reason it was decided that a better study of the action of the radiations on the corpuscles could be made by tracing the gradual increase with time of the completely hæmolysed corpuscles, i.e., the so-called ghosts, than by a quantitative measurement of the amount of hæmoglobin liberated.

From each sample of blood slides were prepared, and by means of a Thoma-Zeiss apparatus a count was made of the total number of red corpuscles per c.mm. and of the relative number of ghosts.

When hæmolysis was complete the corpuscles were found to be colourless and slightly shrunk, but retaining their corpuscular form.

Samples of the blood were removed 2, 6, 19, and 42¾ hours after exposure to the emanation had begun.

The count of the citrated blood at the beginning showed 2,730,000 red corpuscles per c.mm., and at the end of the exposure 2,690,000, and therefore practically no corpuscles were completely disintegrated.

The data given in Table I show the different stages of the process. Whereas no ghosts were visible till about twenty hours after the exposure had begun, their number subsequently increased very rapidly, a result to be anticipated if the release of the hæmoglobin is a gradual process.

TABLE I.

Time in hours.	Percentage of completely hæmolysed corpuscles.
2	0
6	0
19	7·2
$42\frac{3}{4}$	84·0

The spectrum of met-hæmoglobin was observed at the end of the observations. The conversion from oxy-hæmoglobin was not complete, however, as some of the bands of this substance were also visible.

While watching the process of hæmolysis it was thought advisable to see whether the blood containing the products of the injured corpuscles had any hæmolytic action on those that remained.

For this purpose fresh citrated blood was obtained, which, in a dilution of 1 in 100, gave a count of 214 corpuscles in 80 squares of the Thoma-Zeiss apparatus. The experimental hæmolysed corpuscles were centrifugalised to clear the liquid of the ghosts, and the supernatant fluid added to an equal volume of the freshly-prepared blood. The emanation held in solution by the hæmolysed blood would quickly escape from the liquid on exposure to the air.

A count of the mixed bloods, if no hæmolysis had been induced in the fresh corpuscles, should, of course, give the number 107 in 80 squares; the number obtained was 115, which showed that no immediate action resulted. The mixture was kept for twenty-four hours, and a count again made, which resulted in the number 116, and no ghosts were found. These observations show that the blood after radiation had itself no hæmolytic effect on fresh red corpuscles.

Proof that the Hæmolysis was due to the *a* Particles.

From the results of the foregoing experiments there was little doubt that the hæmolysis observed on exposing blood to the emanation was due to the *a* particles, but the crucial experiment of allowing the blood to be radiated by these particles without the emanation actually coming into contact with it, and thence into solution, had yet to be done to decide the question.

Recently, facilities have been afforded us of performing the experiment fulfilling this condition. Hæmolysis has resulted as a direct result of the α radiation.

The method by which this has been demonstrated is the following:—

A finely powdered specimen of radium bromide was spread over a circular area of 2 sq. cms., this being the bottom of a cavity 1 mm. deep in a brass capsule (Fig. 1). The grains were held in position by very thin varnish. The cavity was covered over with a very thin sheet of mica, A, which was made air-tight with shellac and Brunswick black.[†] The mica sheet was thin enough to allow the α particles to pass through it, without allowing the escape of any emanation.

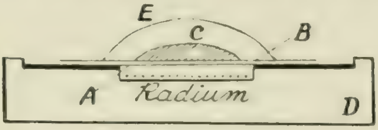


FIG. 1.

Another thin sheet of mica, B, was taken and a drop of citrated blood, C, spread over a known area of it. The drop was covered over with a shallow watch-glass, E, vaselined round its edge, to prevent evaporation. The mica B was then placed over A and allowed to remain in position for any desired interval.

Preliminary observation showed that marked hæmolysis resulted after an interval of twenty-four hours' radiation.

The "range" or distance of travel of the α particles is an accurately known quantity.

The ranges in air of the α particles with which we are here concerned, namely, those emitted by radium, the emanation, RaA and RaC, are 3·50 cms., 4·35 cms., 4·82 cms. and 7·08 cms. respectively.

From the conditions of the experiments, the α particles have to penetrate two very thin sheets of mica before reaching the corpuscles which are distributed over the surface of the second sheet. These two mica sheets will, therefore, cut down the range of the particles by an amount which was determined as follows :—

[†] Two such brass capsules were made, one containing 2·4 mgms., the other 3·27 mgms. $RaBr_2$. We are indebted to Mr. F. H. Glew for their preparation.

A screen of zinc sulphide which showed scintillations when radiated by α particles was mounted on the stage of a microscope, and the radium capsule, with its single mica sheet A which covered it, brought up underneath so as to give scintillations on the screen. Owing to the range of the particles being cut down by this sheet, scintillations could not be observed until the radium was brought up to a distance of 5·53 cms. from the screen. This shows that the first mica sheet had cut down the range of the particles from RaC by 1·55 cms., indicating a thickness of ·006 mm. for the mica sheet. On interposing the second mica sheet B the scintillations were not visible until the radium was distant 4·25 cms., corresponding to a further reduction of 1·28 cms. Thus after traversing the two mica sheets A and B the ranges of the α particles would be equivalent to ·67 cm., 1·52 cms., 1·99 cms. and 4·25 cms. of air.

During the course of this work numerous mica sheets were used, the thicknesses of which were found in the following way :—A polonium preparation was placed inside an electroscope and the ionisation, due to the α particles emitted from it, measured. The mica sheet in question was then placed over the preparation and the reduction in the ionisation noted. Knowing the reduction in the ionisation caused by the sheets of known thickness used in the scintillation experiments, a simple reduction † gave the thickness of the particular mica sheet.

From the given experimental arrangements it is clear that the degree of hæmolysis occurring must depend on the depth of the drop of blood placed on the mica sheet B. If a very thin layer of corpuscles is formed, the α rays may be able to penetrate it completely, and after sufficient time has elapsed hæmolysis may be complete. On the other hand, with a large drop the layer of corpuscles may be too deep for complete penetration by the rays, and despite a long exposure the degree of hæmolysis may still be limited.

In order to find the number of α particles required to completely hæmolyse a red corpuscle, a drop of blood was chosen so that when spread over a known area the depth of the layer of red corpuscles was just greater than the range of the α particles from RaC.

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† Rutherford "Radio-activity," p. 178; 1904.

A series of experiments was therefore conducted in which 12·5 c.mms. of citrated blood (½ citrate) were spread over 1·5 sq. cms. and the percentage of completely hæmolysed corpuscles determined for various times of exposure.

It will be remembered that when the emanation was mixed with blood, free hæmoglobin could be detected long before any completely hæmolysed corpuscles could be found. A sample of blood which has been radiated, say, for a few hours and examined at once, showing no ghosts, might, if kept for some time, exhibit them. Such was found to be the case. Similar volumes of blood (12·5 c.mms.) were spread over similar areas and radiated for two hours. One was examined at once and showed no ghosts. The other was examined twenty-four hours later and 8 per cent. of the corpuscles were found to be completely hæmolysed.

It was clear, therefore, that if an accurate relation between the time of radiation and the number of hæmolysed corpuscles were to be found, sufficient time must elapse after radiation and before the count was made to allow of the release of the hæmoglobin from the affected corpuscles. Twenty-four hours were found to be sufficient for this purpose.

The same volume of citrated blood (12·5 c.mms.) was taken each time and spread over an area of 1·5 sq. cms., on a sheet of mica whose thickness was determined in the manner already described. This was then exposed over the radium, giving out α, β and γ radiation, the first of which was alone responsible for any hæmolysis observed. A watch-glass control was provided in each case.

The variation of totally hæmolysed corpuscles with the time of exposure may be seen from the number in Table II and the curve in Fig. 2.

It will be seen that hæmolysis was not quite complete after twenty-four hours' exposure. This was probably due to the fact that the layers of corpuscles were just too numerous for the α particles to completely penetrate them. It was found that the percentage of completely hæmolysed corpuscles for twenty-four hours' exposure varied inversely as the volume of the drop, the area over which it was spread being kept the same. Three examples may be quoted in which the count was made immediately after the radiation was stopped.

Volume in c.mms.	Percentage of hæmolysed corpuscles.
12·5	60
18·5	40
33·2	21

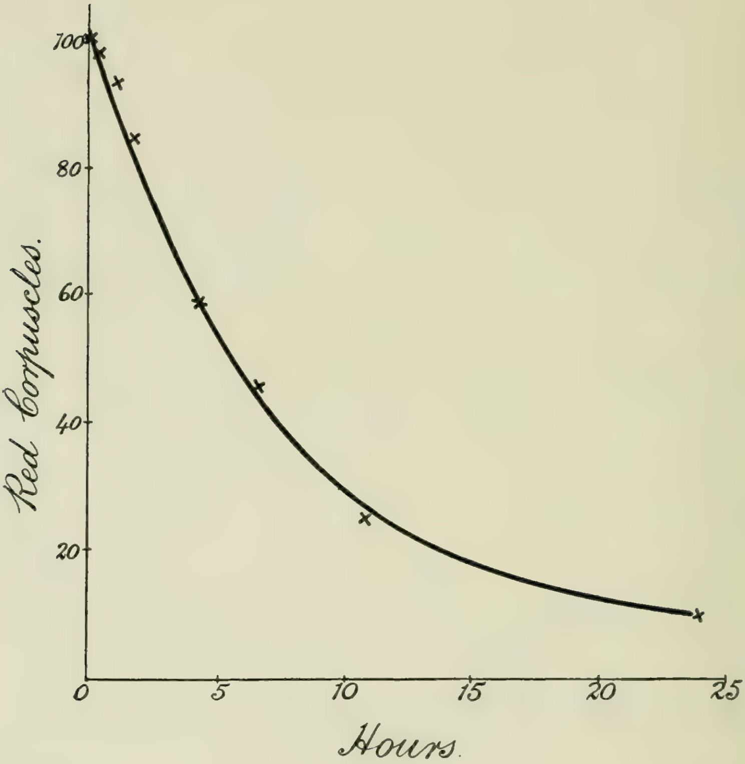

FIG. 2.

TABLE II.

Time of Exposure.	Percentage of unhæmolysed corpuscles.
0 h. 15 m.	98
1 h. 0 m.	93·5
1 h. 40 m.	84·5
4 h. 15 m.	58·8
6 h. 35 m.	45·6
10 h. 45 m.	25
24 h. 0 m.	10

The Number of α Particles required to Hæmolyse a Red Corpuscle.

Such an estimate is possible owing to the precision with which the essential quantities in the calculation are known.

On the one hand the number of red corpuscles in any sample of blood can be readily estimated by means of a Thoma-Zeiss apparatus, and, on the other hand, the number of α particles emitted per second from a measured quantity of radium emanation is known.[†] Two separate estimates may be made of the number in question. From the experiments in which emanation was mixed with the blood, a simple calculation results in the number 2,000 α particles being required for the complete hæmolysis of a red corpuscle. From the experiments in which the α particles had to penetrate two sheets of mica their diminution in range was measured in the way already described. Knowing the number of α particles projected from the capsule per second,[‡] a maximum estimate of the number in question is 8,000.

In view of the different experimental conditions the difference between the two numbers is not significant.

The Action of β and γ Rays on Leucocytes.

From the experiments hitherto recorded it has been seen that the β and γ rays from quantities of $RaBr_2$ of the order 5-10 mgms. give no visible hæmolysis of red blood corpuscles, after exposures lasting two or three days. It was decided to see whether any changes could be detected in leucocytes after prolonged radiation from this order of quantity of radium.

A quantity of citrated blood was centrifugalised, the clear liquid pipetted off, and the mixture of red and white corpuscles exposed to the β and γ radiation from the 20 mg. tube of $RaBr_2$ for various times. No structural alterations were visible, the leucocytes subsequently stained quite well, and were apparently in as good condition as those constituting the control. The phagocytic power of the leucocytes was estimated by adding two volumes of the corpuscle suspension to one volume of an emulsion of Staphylococcus pyogenes aureus. After 15 minutes incubation films were made and counted with the following results:—

[†] Rutherford and Geiger, "Proc. Roy. Soc." 1908, p. 173.

[‡] We have pleasure in thanking Mr. E. Marsden, B.Sc., for this determination.

Time of Radiation.		Micro-organisms in 100 Leucocytes.
24 hours.	Experimental Film gave	583
	Control	489
43½ ,,	Experimental Film gave	195
	Control	220
42 ,,	Experimental Film gave	292
	Control	250
40 ,,	Experimental Film gave	156
	Control	157

It is thus seen that the β radiation corresponding to 5·7 mgms.† and the γ radiation from 20 mgms. $RaBr_2$, acting for about two days, have a very small action, if any, on leucocytes *in vitro*.

The Action of the α Rays on Leucocytes.

It has been found that the α particles are capable of not only destroying the leucocytes, but also, by virtue of their action on the serum, rendering a radiated region free of leucocytes.

A simplification of Ponder's method of obtaining leucocytes has been used for these experiments.‡

A drop of blood is put on a mica plate, covered, but not touched, by a watch glass to prevent evaporation, and incubated at 37°C. for about 20 minutes. On removal of the clot, large numbers of leucocytes are found on the surface of the mica, to which they adhere firmly, for they may be repeatedly washed and then stained without being freed.

If the blood be not incubated the clotting is delayed and the motion of the leucocytes to the surface considerably prolonged; this is of importance in some of the experiments about to be described.

A piece of mica allowing easy penetration of the α particles was taken, immersed in absolute alcohol for a few minutes, and then dried over a flame. A drop of blood was then placed on it, covered with a watch glass, and placed over the radium capsule.† The area from which the rays came was a circle of 2 sq. cms., and the drop of blood was spread over a smaller area to ensure uniformity of radiation.

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† See Footnote, page 129.

‡ Ponder, "Proc. Cam. Phil. Soc." 1909, XV. pt. 1.

At the end of about 20 hours' radiation at room temperature the clot was removed from the mica surface, which was then washed with saline and stained. Practically no leucocytes were found on the mica, while a control showed large numbers of them. This suggested that the leucocytes did not come down on to the surface which was at the time subjected to the radiation.

To test this, a drop of blood was placed on a thin sheet of mica, but before placing it on the mica window of the Ra capsule one half of the window was covered with a screen thick enough to completely prevent any α rays getting through it. The sheet of mica carrying the drop of blood was now placed over this system, which ensured that while

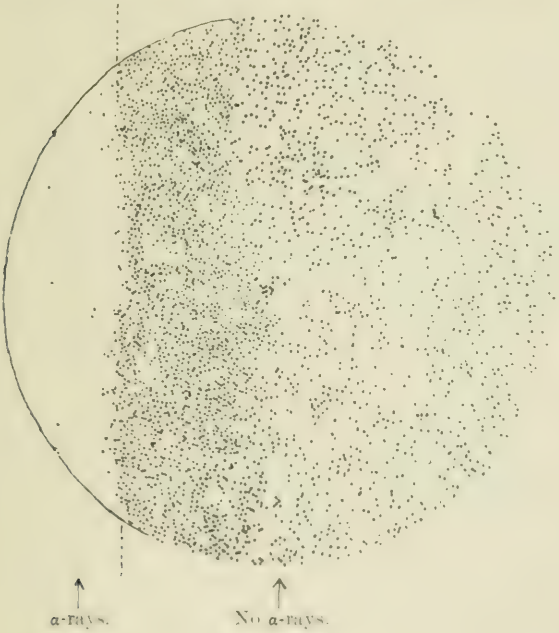

FIG. 3.

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† Two capsules were in use, one containing 2·4, the other 3·27 mgms. $RaBr_2$. It is unnecessary to specify on each occasion which one was used, as the following observations are not quantitative.

one half of the drop received α radiation, the other half received none. The radiation continued for about 20 hours at room temperature, when the clot was removed, the mica surface washed with saline and stained. The picture presented was as shown in Fig. 3.

It will be seen that the half of the field which had been subjected to α rays was practically devoid of leucocytes, the other half presenting them in large numbers. The line of division was quite sharp, and inspection showed moreover an accumulation of the leucocytes near the border, suggesting that there had been a movement of the leucocytes out of the radiated region into one which was free of the α rays.

This experiment was repeated under practically identical conditions, with the same result, the accumulation at the border being again evident.

The results bear a striking pictorial resemblance to those obtained by one † of us, in which by covering one half of the top surface of a glass cell with a layer of micro-organisms, the leucocytes were found in larger numbers on this half than on the other. This result has not yet received an explanation, but in the experiments with which we are here concerned there are two possibilities open. Either the leucocytes do not come down on to the radiated surface, or they are destroyed *in situ*.

The two following experiments provided contrasts to those just described :—

A drop of blood was shed on a thin sheet of mica, and immediately placed in the incubator at 37°C. for two hours. These conditions ensure a plentiful swarm of leucocytes on to the mica surface. It was then removed from the incubator and placed over the Ra capsule, one half of the window of which was again screened to prevent α rays emerging. The conditions then were that large numbers of leucocytes were already firmly fixed on the mica surface, one half of which encountered at once the α rays. After an exposure of about 20 hours the clot was removed, the surface carefully washed with saline, and stained. The half protected from α radiation contained numerous leucocytes, the other half, however, was partially covered with leucocytes in an advanced stage of degeneration as evidenced by lack of nuclear staining and almost complete disintegration of many of them.

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† H. Chambers, "Arch. Middlesex Hospital," Vol. XIX., 1910.

Presumably, owing to their initial fixation to the mica surface, they retained their position in spite of the radiation. No accumulation at the border of the protected region was observed; if anything, a diminution of their numbers had occurred there. In the experiment described, in which directly the blood was shed it was exposed to the radium at room temperature, the film showed extremely few degenerate leucocytes in the radiated region, thus suggesting that in this case they had not come down on to the radiated surface. The accumulation at the border suggests that they had moved into the protected region. These impressions are confirmed by experiments to be described later.

The leucocytes do not, however, move as a direct result of the α radiation, for if incubation occur simultaneously with radiation, leucocytes are found on the radiated surface, although to a modified extent.

The motion of leucocytes out of the clot is delayed at room temperature, and during this process changes are taking place in the radiated serum that forms a layer over the mica. As will be seen later, there is a lowering of the opsonin and complement content of the serum over one-half of the mica surface, that over the other half, except for a small amount of diffusion, remaining constant.

The leucocytes seem to move from a radiated to a nonradiated region, i.e., from a serum in which changes have been induced by the radiation, to a serum which is unaltered. This motion can be explained by changes in surface tension corresponding to changes in the concentration of the fluids in which the leucocytes are moving.

If, however, the process be accelerated by incubation, there will be comparatively small changes in the serum; the leucocytes collect on the mica in spite of the radiation, perhaps to a modified extent, and are then gradually destroyed *in situ*.

The surface tension of radiated serum, determined by the capillary-tube method, showed a reduction when compared with normal serum.

The change is indicated by the figures in Table III.

TABLE III.—SURFACE TENSION IN DYNES PER CM. AT ABOUT 10° C.

Normal Serum.	Radiated Serum.	Time of Radiation.
66·2	63·2	24 hours
66·2	61·2	44 ,,
67·5	59·1	54 ,,

The Destruction of Leucocytes by α Rays.

It will be seen later that marked degenerative changes were observed in leucocytes which had been exposed for some hours to the action of the emanation. This degeneration has been a very marked feature of some of the experiments just described, and has been attributed to the direct action of the α particles.

A drop of blood was shed on to a thin mica sheet, covered with a watch glass, and then placed on the radium capsule, the radiation from which was screened in such a manner that it was entirely confined to a very small square window of about 1·5 sq. mm. On placing the mica sheet over the capsule, therefore, the drop of blood was not radiated by the α rays, except that portion of it which was directly above the small window; the area radiated was a little larger than the window, owing to a small amount of cross-firing.

After an exposure of about twenty hours at room temperature the clot was removed, the mica surface washed with saline and stained. The resulting picture was as indicated by Fig. 4, from which it is seen that in a region corresponding to the radiated area there is an almost complete absence of leucocytes; the area free of leucocytes is, however, slightly greater than that of the window, the ratio determined by a magnified projection on squared paper being 1.34 : 1. This increase is probably on account of the cross-firing of the rays.

This experiment is a variation of that previously described in which the α radiation from the circular aperture of the radium capsule was confined to a semi-circle.

The following alteration in the initial procedure gave an interesting contrast:—

A drop of blood was shed on to a thin mica sheet, covered with a watch-glass, and incubated for one and a half hours,

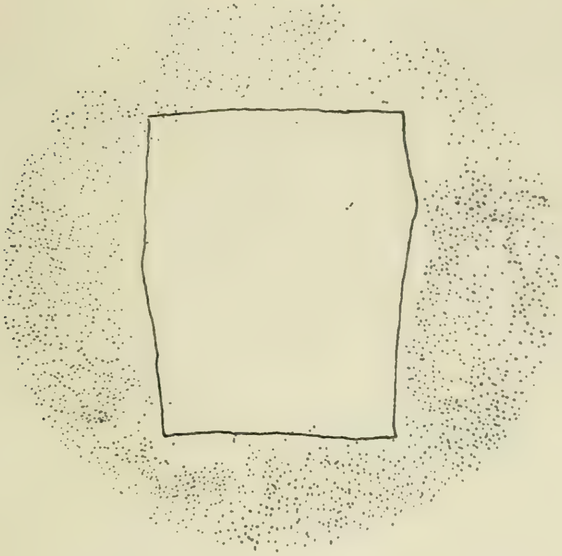

FIG. 4.

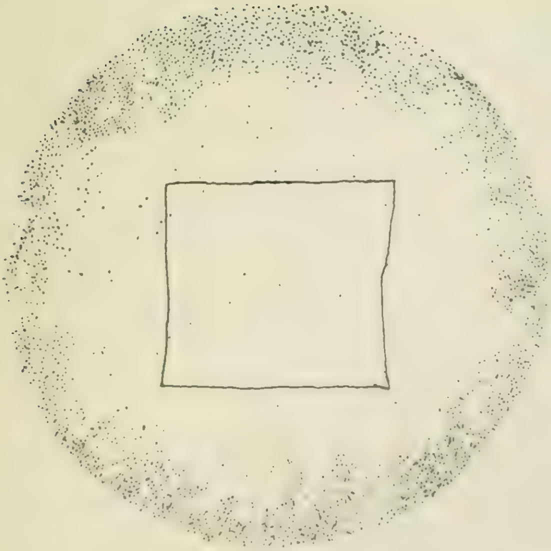

FIG. 5.

thus ensuring a plentiful supply of leucocytes on the mica surface.

The system was removed from the incubator and placed over the radium capsule, which was again provided with a small square window of area 1 sq. mm., through which came the α rays. After an exposure of about twenty hours at room temperature, followed by the usual staining process, the picture presented was as shown in Fig. 5.

Inspection at once showed that the area free of leucocytes was much larger than that of the window. A measurement similar to that already described gave the ratio 2.8 : 1.

If, therefore, the α radiation proceeds during the slow migration of the leucocytes out of the clot at room temperature, the area free of leucocytes practically corresponds to the aperture through which the rays come.

If, however, the leucocytes are first allowed to make their way to the mica surface, part of which is then radiated, the area free of leucocytes is found to be much larger than that of the radiated area, the times of exposure in the two cases being the same.

The suggestion is put forward that the leucocytes which have been destroyed, after making their way to the mica surface, liberate some fluid products which have a destructive effect upon the leucocytes in the surrounding zones. These products gradually diffuse away from the radiated area and effect the destruction of leucocytes quite outside the direct stream of the α rays.

It seems clear that any change in the serum alone cannot account for the differences in question, for if an enlargement of the area occurred in one case, there seems no adequate reason why it should not have done so in the other.

The experimental effects can, on these lines, only be explained if the leucocytes do not come down to anything like the same extent on to the radiated area, for otherwise similar results would be obtained in the two cases.

The following experiments have been made to substantiate the effects and to test the explanation suggested :—

The procedure already indicated was followed with three different sized square apertures. In one series the radiation operated directly the blood was shed and allowed to clot at room temperature, and in the other series the blood was first incubated for one and a half hours to obtain the leucocytes on the mica surface, after which radiation commenced. In both cases the radiation lasted about twenty hours.

Table IV. shows the results obtained.

Area of Aperture.	Radiation without incubation. Ratio of Area free of Leucocytes to Area of Aperture.	Radiation after incubation. Ratio of Area free of Leucocytes to Area of Aperture.	
1 sq. mm.	1·46	3·0 2·8	Mean 3.05
1·5 ,,	1·38	2·4 2·0	Mean 2.20
5 ,,	1·11	1·32 1·24	Mean 1.28

The numbers in the second column show the effect of cross-firing, this being more apparent for a small aperture than for a large one. The numbers in the third column show the diffusion effect, which should cause the ratio to decrease as the aperture is increased.

The Action of the Radiations on Opsonin.

Experiments have been described in which citrated blood was exposed to the action of the emanation in closed glass bulbs, and the degree of hæmolysis observed from time to time. Simultaneously with these observations the opsonic content of the blood was compared with that of the control.

The usual procedure in estimations of opsonin was adopted and an emulsion of Staphylococcus aureus was used in every case. In one of several experiments a glass bulb of 30 c.cs. capacity was filled with 26·5 mgms. of emanation and 2 c.cs. of citrated blood. The first determination, which was made nineteen hours after exposure of the blood to the emanation had begun, gave evidence of a reduction in the opsonic content as measured by the relatively small microbic content of the leucocytes. These leucocytes had, however, been subject to the radiations, and were noticed to be much degenerated, in consequence of which the radiated blood was centrifugalised, and the opsonin content of the supernatant fluid was determined with freshly-washed leucocytes.

After forty-four hours' exposure the following result was obtained :—

Control fluid gave 372 microbes in 60 leucocytes.
Experimental fluid gave 34 microbes in 60 leucocytes.

The effect, then, of long exposure is apparently a marked reduction in the opsonin.

According to some experiments by H. Reiter, in which emanation was mixed with blood for short periods of time, an increase of phagocytosis was observed, for, to quote from this author, " Soweit nach den Versuchen in vitro zu erteilen ist, scheint die Emanation die phagocytäre Tätigkeit der Blutzellen anzuregen, in einzellen Fällen bis zu 30 %." †

The experiment was varied, as the objection might be raised that during the previous exposure the serum was the recipient of products of radiated corpuscles, the action of which might possibly injuriously affect phagocytosis.

Some citrated blood was centrifugalised, and about 1 c.c. of the supernatant fluid was let into a bulb of volume 7·8 c.cs. containing 9·15 mgm. of emanation, and examined on two occasions with the following results :—

Experimental fluid gave 84 micro-organisms in 160 leucocytes
Control ,, ,, 495 ,, ,, ,,
(Time of exposure, 19 hours).
Experimental fluid gave 57 micro-organisms in 100 leucocytes
Control ,, ,, 388 ,, ,, ,,
(Time of exposure, 42 hours).

These observations again suggest a marked reduction in the opsonin of the radiated fluid.

In order to test whether there were substances formed in the radiated fluid which might be inhibitory to phagocytosis further observations were made with the two plasmata of the last experiment. The procedure is indicated as follows :—

Experimtl. plasma +	Control plasma +	Micro-organisms +	Washed leucocytes — Film I.
1 volume	1 volume	1 volume	2 volumes

Saline solution + Control plasma + Micro-organisms + Washed leucocytes — Film II.

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† H. Reiter, " Zentralblatt für Röntgenstrahlen, Radium," etc., 1910, p. 243.

A count of the two films which were prepared gave the following result:—

Film I. gave 441 micro-organisms in 150 leucocytes.
,, II. ,, 541 ,, ,, ,,

It is clear that if the experimental plasma had contained substances inhibitory to phagocytosis their influence should still be manifest when added to the control plasma. The difference between the counts of the two films, amounting as it did to only 20 per cent., shows that the large reduction previously observed with the radiated serum is mainly to be attributed to a reduction in the opsonin normally present.

To find which of the radiations the effect could be attributed to, 9·76 mgms. of emanation were inclosed in a very thin glass tube, the walls of which were sufficiently thick to exclude the α radiation while allowing free exit of the β and γ rays. This tube was then surrounded by another containing $1\frac{1}{2}$ c.cs. of citrated blood. Thirty-seven hours after the exposure had begun opsonic measurements on the fluid were made with freshly-washed leucocytes and gave the following results:—

Experimental fluid gave 474 micro-organisms in 60 leucocytes
Control ,, ,, 415 ,, ,, ,,

This difference is about the possible experimental error of the count on such a number of leucocytes.

The experiment was repeated under slightly different conditions by exposing citrated blood to the β and γ rays from 20 mgms. of pure $RaBr_2$ contained within a thin glass tube.* After forty hours' exposure the following results were obtained:—

Experimental fluid gave 258 micro-organisms in 60 leucocytes
Control ,, ,, 214 ,, ,, ,,

The difference is again what might be due to experimental error, but in view of the fact that the differences in the two experiments went the same way, it was decided to repeat the experiment using blood serum, part of which was exposed to

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* The walls of the glass tube cut down the β rays to just less than one-third of their equilibrium value. The β radiation coming through the tube was equal to that from 5·7 mgms. $RaBr_2$.

20 mgms. $RaBr_2$ in the manner just described, the remainder being kept as control.

Measurements made 42½ hours after exposure had begun gave the following results :—

Experimental serum gave 419 micro-organisms in 60 leucocytes
Control ,, ,, 407 ,, ,, ,,

The above experiments indicate that with the order of quantity (5–10 mgms.) of radium bromide used, the β and γ rays have little, if any, effect on opsonin for the above times of exposure.

The inference was that the effects obtained with the emanation were due to the α radiation, and this has been proved to be the case as follows :—

The experiments were made with the apparatus already described (Fig. 1), the usual procedure being to expose a small quantity of serum to the α radiation coming up through the mica window from the radium in the capsule (the β and γ radiations were also present, but these have been seen to be without effect). The experimental details were very similar to those obtaining in the experiments on the red corpuscles, which have already been described and need not again be detailed.

A series of observations was made by taking a measured volume of serum (12·5 cms.) spread over an area of 1·5 sq. cms. on one of the thin mica sheets and exposing it to the radiation from one of the capsules (3·27 mgms.). A fresh sample of serum was used for each exposure, as owing to the very limited penetration of the fluid by the α rays, only just sufficient for an opsonic determination was radiated. Had a larger volume of serum been used the percentage reduction of the opsonin would have been diminished. The mica sheets were selected as nearly as possible of the same thickness. After the serum had been radiated for any selected interval its opsonic content was compared with that of the control.

The numerical results obtained are given in Table V., and shown graphically in Fig. 6.

It will be seen from the numbers given that after a radiation lasting forty-five hours the opsonin had almost completely disappeared from the serum.

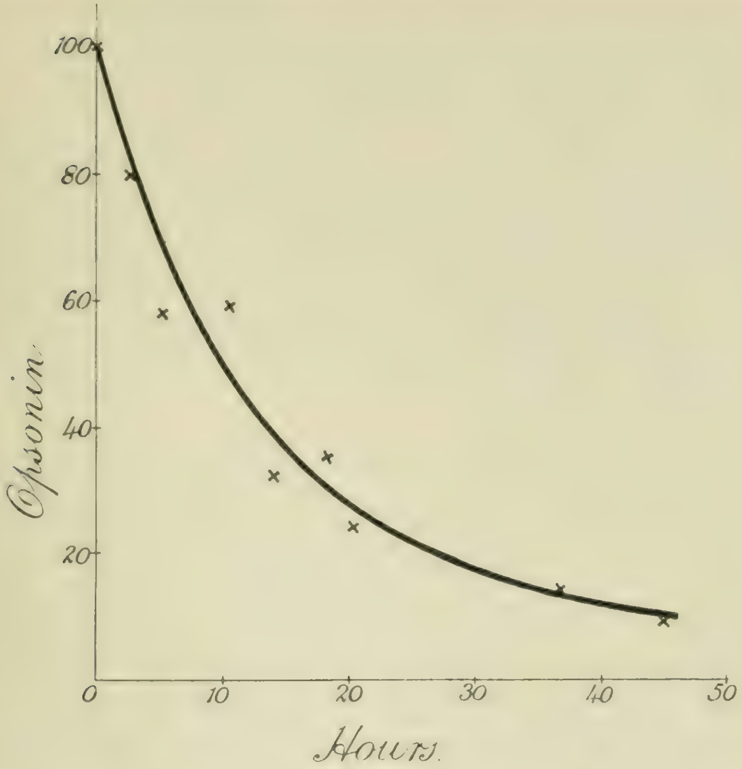

FIG. 6.

TABLE V.

Time of Radiation.	Percentage of Opsonin in Radiated Serum.	Number of Micro-organisms in 100 Leucocytes.	
		Control.	*Experiment.*
2h. 40m.	80	620	506
5h. 15m.	58	620	360
10h. 30m.	59	386	228
14h. 0m.	31 42*	430	182
18h. 20m.	35 47*	507	244
20h. 15m.	24	210	50
36h. 45m.	14	383	53
45h. 0m.	9	681	64

* A smaller quantity of radium was used in these cases; the numbers on reduction give 31 per cent. and 35 per cent.

The reduction observed after forty-five hours' exposure to α radiation alone is of just about the same magnitude as that recorded when 2 c.cs. of serum were mixed with emanation, giving an almost identical intensity of α radiation.

The general character of the curve is of the simple exponential type, and indicates that the amount of opsonin changed during any small interval of time is roughly proportional to the amount then present in the serum.

Owing to the possibility that the method of estimating the amount of opsonin is not strictly quantitative, it cannot be asserted that the destruction of opsonin rigorously follows an exponential law.

The Action of the Radiations on Complement.

A similar series of observations was made with hæmolytic complement. For this purpose a thin film of serum was exposed to the α radiation from one of the capsules in the manner already described. The complement in the radiated serum was then compared with that contained in the control.

In order to obtain a quantitative estimate of the complement a method similar to that described by Dr. Emery * has been used.

Briefly, the method consists in procuring a 20 per cent. emulsion of fully-sensitised red blood corpuscles, obtained by adding to one volume of washed corpuscles four volumes of a strong immune serum. To four volumes of this emulsion is added one volume of the serum, the complement in which is being tested. After being kept at 37° C. till maximum hæmolysis under the conditions has occurred, the liquid is centrifugalised and the amount of free hæmoglobin in a constant volume (76 c.mms.) of the supernatant fluid tested by means of a Sahli hæmometer.

This instrument was calibrated by adding to four volumes of sensitised red corpuscles one volume of serum of varying dilutions. The amount of free hæmoglobin, as read from the scale of the instrument, was found within the limits of the

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* Emery, *Lancet*, February 25th, 1911, p. 490.

experimental error to be proportional to the strength of the serum added, and therefore to the amount of complement present, as may be seen from Fig. 7. Accuracy, however, is not claimed for hæmometer readings below 20.

The volume of serum exposed to the radiation in the opsonin experiments was 12·5 c.mms., but this was found to be an inconveniently small quantity in the complement estimations.

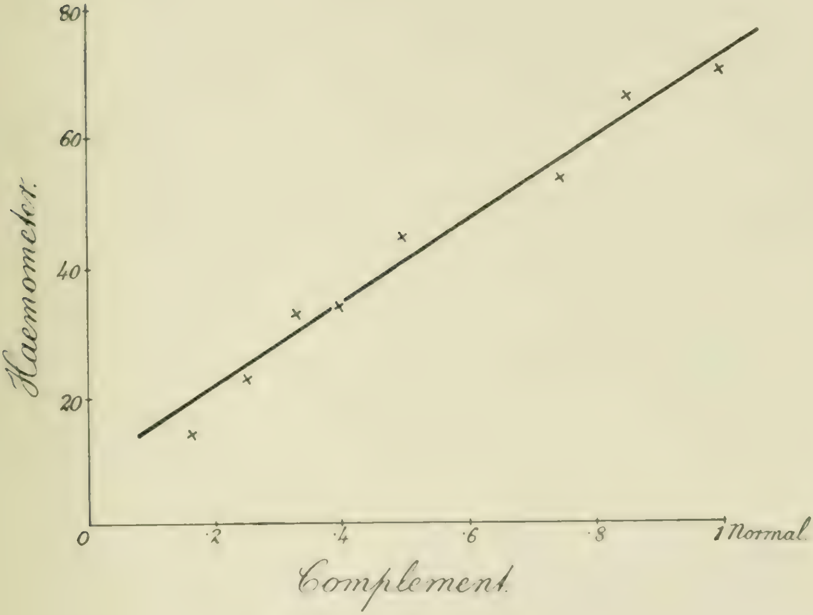

FIG. 7.

Accordingly the volume was increased to 30·5 c.mms., and this was spread over a circular area of 2 sq. cms. on the mica sheets.

After an exposure of any desired interval the amount of complement in the radiated serum was compared with that in the control. The results may be seen from Table VI. and Fig. 8.

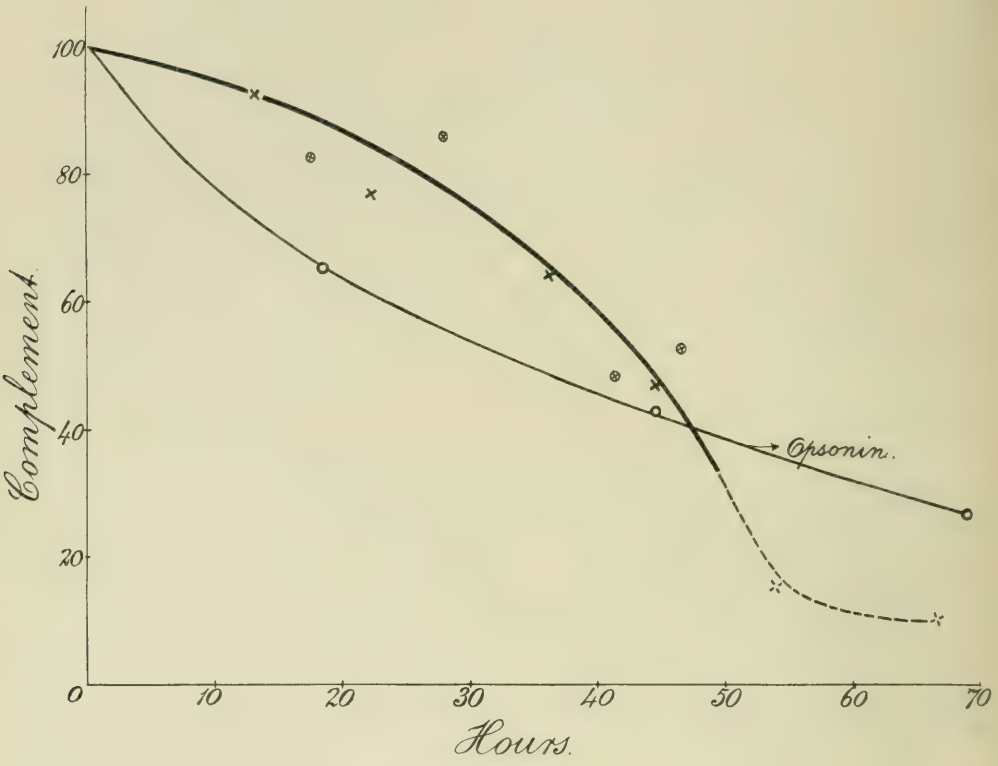

FIG. 8.

TABLE VI.

Time of Exposure.	Percentage of Complement remaining.
13h. 15m.	92
17h. 30m.	82·5
22h. 15m.	~~67·6~~ 76·6
28h. 0m.	86
36h. 25m.	64
41h. 15m.	48
44h. 35m.	46·8
46h. 30m.	52·9
54h. 15m.	15 estimated
66h. 30m.	10 ,,

A comparison between this curve and that in Fig. 6 shows a striking dissimilarity in the reduction of opsonin and complement in serum when subject to α radiation; but the two curves are not strictly comparable, owing to the difference in the volume of serum radiated in the two cases. For this reason three determinations of the reduction in opsonin were made in which the same volume of serum was radiated as in the complement experiments. These three observations are indicated by the thin line curve (Fig. 8), the type of which is identical with that previously obtained, the only difference being a diminished rate of reduction of opsonin, as was to be anticipated.

Inspection of the complement curve shows that the reduction is slow at first, the rate gradually increasing with time, as may be seen from the convexity of the curve to the time axis.

The experimental and control sera were, except for the radiation, kept under identical conditions at room temperature (8°–12° C.). The spontaneous disappearance of complement is therefore eliminated as a disturbing factor by a comparison between the two sera.

It should be pointed out, however, that fresh serum was used for every observation, because it was found that the complement in serum which has been kept for some days at 0° C. was more affected by the radiation than if the serum were fresh.

The general character of the two curves (Fig. 8) indicates the separate identity of opsonin and complement. The complement curve suggests either that this substance becomes more unstable under the action of radiation or that its amount is in some way dependent upon some other substance present in the serum.

The small initial reduction in complement could, on the latter supposition, be explained if this substance, under the action of the radiation, were eventually reduced to complement; then, despite the simultaneous reduction in the latter, a supply would be provided owing to the breaking down of the former.

Our present methods need elaboration before this question can be settled.

SUMMARY OF CONCLUSIONS.

1. Red blood corpuscles are hæmolysed by the action of *α* rays, and oxy-hæmoglobin is converted into met-hæmoglobin.

2. Leucocytes undergo marked degenerative changes when subjected to *α* rays.

During the process of clotting leucocytes appear to move away from an *α*-radiated region. This movement has been attributed to changes found to occur in the surface tension of blood serum when radiated.

3. The specific properties of opsonin and hæmolytic complement are lost when serum is exposed to *α* rays. The progressive changes caused by these rays indicate the separate identity of opsonin and complement.

4. The *β* and *γ* rays have yielded negative results in analogous experiments.

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